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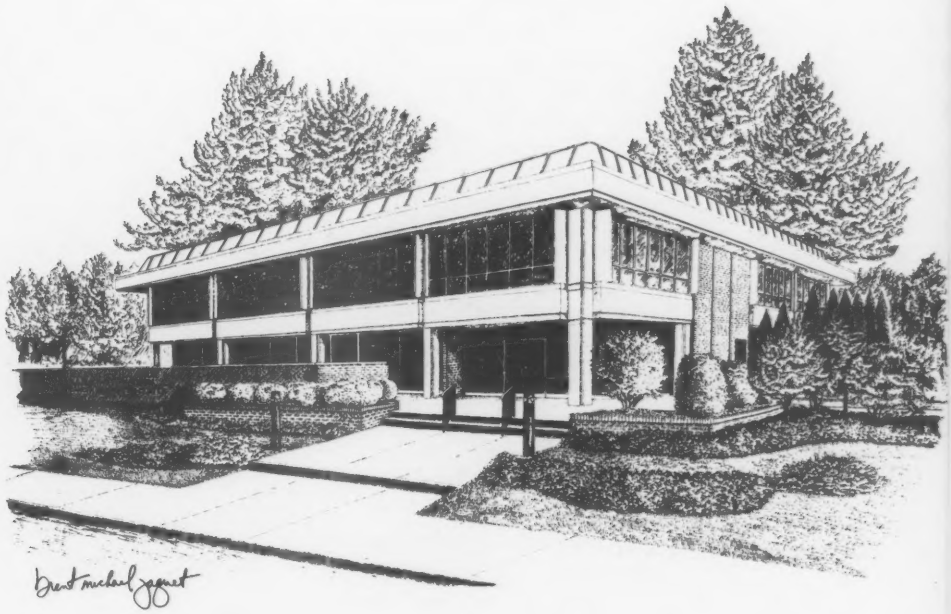

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Jean Griffin Baum
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1201 ANNOUNCEMENTS

Role of Adjuvant Chemotherapy in the Management of Patients With Soft Tissue Sarcomas[1]

John H. Edmonson[2,*]

During the last decade several controlled clinical trials of adjuvant chemotherapy have been performed for the purpose of improving the treatment of adult patients with soft tissue sarcomas. An early randomized study of postoperative adjuvant treatment of childhood rhabdomyosarcomas by Heyn et al (1) had produced disease-free status at 2 years in significantly more of the patients receiving adjuvant vincristine and dactinomycin plus radiation therapy as compared with the children receiving only radiation therapy to the tumor bed. Currently, combination chemotherapy is an established part of the treatment for childhood rhabdomyosarcomas, with a 2-year disease-free survival expectation of > 85% in completely excised localized cases even without postoperative radiation therapy (2). Unfortunately, most soft tissue sarcomas in adults are not especially drug-sensitive tumors, and even rhabdomyosarcomas seem not to be as easily controlled in adult patients as in children (3). Thus, the childhood sarcoma model fails to provide direct guidance toward the successful adjuvant chemotherapy for adult soft tissue sarcomas.

With the introduction in 1970 of doxorubicin came hope and enthusiasm for the possible chemotherapeutic control of soft tissue sarcomas in adults, since this drug was found to be more effective than most older single agents in the management of patients with advanced disease. A controlled clinical trial including this drug soon was started at the M. D. Anderson Hospital comparing postoperative radiation therapy plus adjuvant chemotherapy with postoperative radiation therapy alone (4). Monthly courses of vincristine, cyclophosphamide, and doxorubicin were given until, after seven courses, dactinomycin replaced doxorubicin for further adjuvant treatment. When after 18 months of follow-up the two groups were preliminarily compared, 85% of the control group were still free of metastasis compared to only 67% of the chemotherapy-treated group. This disappointing result led to early termination of the study. Of some interest, however, was the observation that patients participating in this study survived significantly longer than had M. D. Anderson patients previously.

Following that initial effort by Lindberg et al (4) in Houston, several other controlled trials have been initiated to identify possible effective adjuvant chemotherapy for soft tissue sarcomas. Perhaps the best known of these is the National Cancer Institute Clinical Center study which was begun in 1977 (5). These investigators superimposed their evaluation of postoperative cyclophosphamide, doxorubicin, and high-dose methotrexate in combination on their continuing randomized study of limb-sparing surgery plus irradiation versus amputation for extremity lesions. Patients who had received by prior random selection limb-sparing surgery plus irradiation then randomly received either adjuvant chemotherapy or follow-up alone. Those patients who had received amputation by prior random selection, however, all received adjuvant chemotherapy. A third category of patients with extremity lesions (those who had not participated in the previous surgical randomization) were also randomly allocated to receive or not to receive adjuvant chemotherapy following primary surgical therapy selected individually by the patients. According to interval analyses of these combined results, the durations of disease-free status and survival have both been significantly longer for those patients with extremity lesions who received the adjuvant chemotherapy than for those who did not. These results have led Rosenberg et al to advise that postoperative patients with soft tissue sarcomas of extremity origin should receive adjuvant chemotherapy. Somewhat surprisingly, a concomitant randomized study of similar postoperative patients whose sarcomas had been excised from head and neck or trunk sites revealed no therapeutic advantage for patients receiving this same adjuvant chemotherapy program. With > 10% of the patients reported to have developed overt congestive heart failure when full doses of the National Cancer Institute regimen were used and nearly one-half of the treated patients showing evidence of cardiac dysfunction on the basis of radionuclide cardiac scans, this regimen has been judged too toxic by the Clinical Center investigators and it is now being compared with a lower dose combination in a subsequent study.

[1]Received July 11, 1983; accepted Aug 8, 1983.

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Another controlled trial begun at the Mayo Clinic in 1975 has also been reported in preliminary form (6). This trial compared postoperative adjuvant chemotherapy (alternating courses of vincristine, cyclophosphamide, and dactinomycin with vincristine, doxorubicin, and dacarbazine) with observation alone by random selection. Control patients who relapsed locally or by distant metastasis later did receive the chemotherapy after surgical excision of the locally recurrent or metastatic tumor. We have reported that the patients who initially did not receive adjuvant chemotherapy have experienced significantly more distant metastases but no more local recurrences than those who did. When last analyzed, the group that did not initially receive adjuvant chemotherapy had required a total of 17 thoracotomies for the excision of metastatic disease compared with three thoracotomies for the treated group with equal survival experience. Salvage surgery for the removal of local recurrences has been equal for the treated and untreated groups. The Mayo study included a heterogeneous population of nonosseous sarcomas of which approximately 80% were extremity somatic soft tissue sarcomas.

Still another controlled trial of adjuvant chemotherapy has recently been reported from Boston (7). Antman et al (7) have randomly treated one-half of their patients with soft tissue sarcomas using doxorubicin at high doses until 450 mg/m^2 was received. An interim analysis of this study has revealed no definite therapeutic benefit from this sometimes lethal regimen. The Boston study also included a heterogeneous population of sarcomas, most of which were extremity somatic soft tissue tumors. One additional controlled trial of doxorubicin as a single agent has been performed by the Eastern Cooperative Oncology Group. This study has been preliminarily evaluated and no signs of benefit from adjuvant doxorubicin treatment have emerged (H. J. Lerner, personal communication). Members of the European Organization for Research on Treatment of Cancer are currently evaluating the effectiveness of vincristine, cyclophosphamide, doxorubicin, and dacarbazine in combination in a controlled clinical trial seeking a large heterogeneous population of primary sarcoma patients (8). This European adjuvant chemotherapy trial has not yet been published. In this country a large Intergroup Adult Soft Tissue Sarcoma Study has recently begun testing single-agent doxorubicin again as adjuvant therapy of primary somatic soft tissue sarcomas, hoping to demonstrate some therapeutic benefit which has not been apparent in smaller studies.

When published reports of survival experience in patients with soft tissue sarcomas treated during the last 30–40 years are compared by date of primary surgical treatment, it appears that the overall prognosis for these patients has been gradually improving (table 1). While these collected institutional series reports may lack the precision and completeness of population-based cancer registry data, the favorable trend in prognosis seems too powerful to be explained by bias in case selection or by reporting variability. This favorable change has occurred during an era of rapid evolution in medicine and society, generally with broad progress in factors affecting both diagnosis and treatment, and we cannot easily determine how much one or another diagnostic or therapeutic maneuver might have contributed to it. Some conclusions, however, can be suggested. The basic trend began before adjuvant chemotherapy had been introduced with several collected surgically treated series yielding 5-year survival rates in the 60% range for patients treated predominantly during the 1960s. Thus, much of this improvement over time clearly is attributable to factors other than adjuvant chemotherapy. Results reported from patients treated during the last decade have been almost uniformly excellent in comparison with previous periods, with 5-year survival rates of 70%–80% recently observed in several institutions. These most recent excellent survival rates have in some reports involved patients who did not receive adjuvant chemotherapy, and with the exception of the patients with extremity lesions in the National Cancer Institute Clinical Center study, control patients appear to be surviving about as well as their adjuvant-treated cohorts. Interval analysis of the Mayo Clinic study has indicated that at least for the initial 5 years following primary surgical treatment, effective salvage surgery may be an important recent addition to the therapy available for patients with soft tissue sarcomas. We and others have observed excellent survival rates following secondary surgery for local recurrences in contrast to the expected treatment failure and fatality described in the older literature (9). In fact, patients who entered our controlled clinical trial following secondary surgery for locally recurrent disease are surviving almost as well as those who entered the study following initial definitive surgery. While somewhat less predictable, the excision of pulmonary metastases also seems to be contributing substantially to the survival of patients with soft tissue sarcomas. Currently, 20%–35% of such patients survive at least 5 years following thoracotomy (10).

It seems that we may have reached the point at which three-fourths of the patients with surgically accessible soft tissue sarcomas may expect to survive their tumors without initial adjuvant chemotherapy, but with the understanding that further salvage surgery and later chemotherapy may be required. While the use of adjuvant chemotherapy following primary surgery may delay or perhaps even prevent some distant metastases and some of the need for secondary surgery, this alternative cannot yet be recommended as being generally effective in this role. Some of these current regimens expose patients to considerable risk of toxic injury which may counterbalance any presumed reduction in salvage surgery requirements. The antisarcoma effects of some adjuvant chemotherapy regimens do appear to be biologi-

TABLE 1.—Survival of patients surgically treated for soft tissue sarcomas

Investigator(s) (institution)	No. and type (date of cases)	Type of primary treatment	% survival at 5 yrs	Reference
Hare and Cerny (Los Angeles Tumor Institute)	194, various ages and sites (1925–1956)	Various surgical ± irradiation	51	Cancer 16:1332–1337, 1963
Cantin et al (Memorial Sloan-Kettering Cancer Center)	653, various ages; trunk and extremities (1935–1959)	Various surgical	39	Ann Surg 168:47–53, 1968
Martin et al (M. D. Anderson)	183, various ages and sites (1945–1963)	Various surgical	40	Soft tissue tumors. *In* Tumors of Bone and Soft Tissue. Chicago, Yearbook Medical Publishers, 1965, pp 333–347
Ferrel and Frable (Medical College of Virginia)	92, various ages; trunk and extremities (1950–1965)	Various surgical	57	Cancer 30:475–480, 1972
Russell et al (American Joint Committee on Cancer)	1215, various ages and sites (1954–1968)	Various surgical and other	41	Cancer 40:1562–1570, 1977
Gerner et al (Roswell Park Memorial Institute)	155, adult; trunk and extremities (1950–1972)	Local, wide and radical	50	Ann Surg 181:803–808, 1975
Rantankokko and Ekfors (University of Turku, Finland)	240, various ages; extremities and limb girdles (1960–1969)	Local, wide and radical (20 radiated, no excision)	47	Acta Chir Scand 145:385–394, 1979
Markhede et al (University of Goteborg, Sweden)	97, various sites and ages (1956–1976)	Radical or conservative excision or amputation	59	Cancer 49:1721–1733, 1982
Simon and Enneking (University of Florida)	54; extremities (1957–1973)	Radical excision or amputation; no irradiation or drugs	62	J Bone Joint Surg 58A:317–327, 1976
McBride (M. D. Anderson)	79; extremities (1960–1972)	Melphalan or dactinomycin by intra-arterial perfusion 6 wks prior to tumor excision	65	Arch Surg 109: 304–308, 1974
Lindberg et al (M. D. Anderson)	300, adult; various sites (1963–1977)	Conservative excision + irradiation	61	Cancer 47:2391–2397, 1981
Leibel et al (University of California, San Francisco)	76, adult; extremities and limb girdles (1960–1978)	Various surgical ± irradiation	74	Cancer 50:1076–1083, 1982
Eilber et al (University of California, Los Angeles)	65, various ages; somatic soft tissue of extremities (1972–1979)	Surgical + intra-arterial doxorubicin followed by irradiation, doxorubicin, vincristine, and high-dose methotrexate	70*	Ann Surg 192:431–438. 1980
Sordillo et al (Memorial Sloan-Kettering Cancer Center)	64, adult; various sites (1975–1979)	Wide or local excision ± irradiation + vincristine, high-dose methotrexate, leucovorin, chlorambucil, doxorubicin, dacarbazine, and dactinomycin	> 80*	J Surg Oncol 18: 345–353, 1981

continued

TABLE 1.—*Continued*

Investigator(s) (institution)	No. and type (date of cases)	Type of primary treatment	% survival at 5 yrs	Reference
Edmonson et al (Mayo Clinic)	61, various ages and sites (1975–1981)	Wide excision or amputation ± combination adjuvant chemotherapy	> 80*	Proc ASCO 1:182, 1982
Rosenberg et al (National Cancer Institute)	65, adult; extremity sites (1977–1981)	Amputation or wide excision + irradiation ± cyclophosphamide, doxorubicin, and high-dose methotrexate	> 80*	Surg Clin North Am 61:1415–1423, 1981

* Estimate.

cally real, and theoretically, with some further refinements such treatments could be clinically useful. Demonstration of the possible life-prolonging effects of chemotherapy in patients with soft tissue sarcomas, however, may require cytotoxic drug regimens with more satisfactory therapeutic indices than that characteristic of doxorubicin used as a single cytotoxic agent.

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Prospective Randomized Trials Demonstrating the Efficacy of Adjuvant Chemotherapy in Adult Patients With Soft Tissue Sarcomas[1]

Steven A. Rosenberg[2,3,*]

The natural history of soft tissue sarcomas in adults is characterized by extensive local invasion into surrounding tissues and by early micrometastatic spread to the lungs. As an understanding of the locally invasive nature of these lesions has evolved, effective measures have been developed for achieving local control in most cases. Wide local resections in combination with radiation therapy or radical local or amputative surgery achieves local control in > 80% of patients with extremity and trunk wall lesions, although somewhat less success is achieved in patients with head and neck and retroperitoneal sarcomas. Despite achieving successful local control, most patients with high-grade soft tissue sarcomas have micrometastatic pulmonary disease at the time of primary resection and ultimately develop clinical evidence of pulmonary metastases. Thus, the 5-year survival of adults with soft tissue sarcomas is, in most reported series, < 60%.

A variety of chemotherapeutic agents exhibit activity in patients with metastatic soft tissue sarcomas. Doxorubicin is the most effective single agent in this disease and results in about 30% partial response rates (1). In combination with other agents such as dacarbazine (DTIC) or cyclophosphamide (Cytoxan), objective responses of about 40% can be achieved with about 10% of patients achieving a complete response.

Because local control is readily achieved, the incidence of micrometastatic disease is high, and the disease appears responsive to chemotherapy. Patients with soft tissue sarcomas represent ideal candidates for the evaluation of adjuvant chemotherapy in the period following local surgery. A variety of difficulties exist, however, in performing clinical trials to evaluate the efficacy of adjuvant chemotherapy in patients with soft tissue sarcomas. Soft tissue sarcomas comprise about 0.7% of all adult malignancies. There are about 4500 new cases per year in the United States and it is thus difficult for any single institution to accrue enough patients to perform meaningful clinical studies. Soft tissue sarcomas are far more variable than most other tumor types. There are approximately 50 different diagnoses that comprise the soft tissue sarcomas and pathologists often disagree concerning assignment of the exact cell of origin and histologic grade. In addition, soft tissue sarcomas can occur in any portion of the body. The somatic soft tissues comprise about 50% of the weight of the adult human and these tumors can thus occur in a wide range of anatomic locations. Finally, as staging criteria have changed and refinements in surgical and radiation therapy techniques have evolved, the reported 5-year survival rates of patients with soft tissue sarcomas treated without adjuvant chemotherapy range from approximately 40% to 60% (2–12). This wide variation makes it difficult to evaluate adjuvant chemotherapy regimens in any but prospective randomized trials.

Despite the complexities arising from the diversity of soft tissue sarcomas, two major features must be accounted for in the design and analysis of clinical trials evaluating adjuvant chemotherapy in this disease. By far, the most important prognostic criterion in patients with soft tissue sarcomas is the histologic grade of the lesion (13). Low-grade (grade I) lesions virtually never metastasize and although they can be locally aggressive, these patients do not represent candidates for adjuvant chemotherapy. Grades II and III represent high-grade lesions and most pathologists cannot reliably distinguish between these two grades (13). It is thus essential that adjuvant chemotherapy trials include only patients with high-grade (grades II and III) lesions. In addition, the ability to obtain local control varies greatly, depending on the site or origin of the soft tissue sarcoma. Retroperitoneal sarcomas are far more difficult to excise in to-

[1]Received Apr 4, 1984; accepted Apr 24, 1984.

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[3]The studies reported in this brief review represent the combined efforts of a large number of people. The cooperation of members of the Surgery Branch, Radiation Oncology Branch, Medical Oncology Branch, Pathology Laboratory, Nursing Service, Rehabilitation Medicine Department, Social Work Department, and many other departments in the Clinical Center of the National Institutes of Health was outstanding and is very gratefully acknowledged.

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to than are lesions on the extremities. In the analysis of adjuvant studies, therefore, it is essential that sarcomas at varying sites be analyzed separately.

Results of National Cancer Institute Trials Evaluating Adjuvant Therapy for the Treatment of Patients With Soft Tissue Sarcomas

Beginning in 1975, the Surgery Branch in close collaboration with the Radiation Oncology and the Medical Oncology Branches instituted prospective trials evaluating the efficacy of adjuvant chemotherapy in the management of patients with high-grade soft tissue sarcomas of the extremities, head and neck, trunk wall, and retroperitoneum.

Patients eligible for these protocols had a diagnosis of either round cell or pleomorphic liposarcoma, pleomorphic rhabdomyosarcoma, synovial cell sarcoma, fibrosarcoma, neurofibrosarcoma, leiomyosarcoma, malignant fibrous histiocytoma, or undifferentiated sarcoma. All patients had lesions categorized as grade II or III. Patients eligible for the trials were found to be free of clinical evidence of metastatic disease either in regional lymph nodes or more distant sites following a standard workup including blood chemical analysis, chest x-ray, complete lung tomograms at 1-cm cuts, liver scan, and bone scan. Patients were excluded from the trial if they had received any chemotherapy or radiation therapy prior to referral to the National Cancer Institute or if they had a history of any other malignant disease except for basal cell carcinoma. Patients < 30 years of age with a diagnosis of embryonal or alveolar rhabdomyosarcoma were not included in the protocol.

Definitive therapy of local disease was accomplished by either surgery alone or the combination of surgery plus radiation therapy. Patients with extremity lesions received either amputation or limb-sparing wide local resection designed to remove all gross disease. Patients receiving the latter treatment also received radiation therapy to all areas at risk for tumor spread between the joints proximal and distal to the tumor including all involved muscle groups. The radiotherapy techniques involved in the treatment of these patients have been published in detail (14). In brief, daily treatments at 180–200 rads per treatment were given to a total dose of 4500–5000 rads. The tumor bed was then treated to a total dose of 6000–6500 rads over an additional 2-week period. An essential feature of treatment was the exclusion of a portion of the circumference of the involved extremity from the treatment portal. Treatment was begun as soon as the wound was healed, generally within 2–3 weeks following surgery.

The chemotherapy regimen utilized in the trial evaluating the efficacy of adjuvant chemotherapy is as follows:

Doxorubicin—Day 1 of 28-day treatment cycle, 50 mg/m^2 by iv bolus. Escalate each cycle by 10 mg/m^2 to maximum of 70 mg/m^2 based on bone marrow tolerance.

Cyclophosphamide—Day 1 of 28-day treatment cycle, 500 mg/m^2 by iv drip. Escalate each cycle by 100 mg/m^2 to maximum of 700 mg/m^2 based on bone marrow tolerance.

When maximum cumulative doxorubicin dose of 500–550 mg/m^2 is achieved, switch to six cycles of:

Methotrexate—Day 1 of a 28-day treatment cycle, 50 mg/kg iv over 6 hours. Escalate each cycle by 50 mg/kg to maximum dose of 250 mg/kg. Leucovorin at a dose of 15 mg/m^2 iv 2 hours after completion of methotrexate and then every 6 hours for eight doses. Discontinue methotrexate after six cycles.

Patients who received the chemotherapy in conjunction with radiotherapy received the first chemotherapy dose 3 days prior to the first dose of radiation. The second dose of chemotherapy was given during the radiation therapy treatment and the remaining doses of chemotherapy were administered after radiation therapy was finished.

All patients in these clinical trials were followed at 2-month intervals with complete physical examination and chest x-rays. Whole-lung tomography was obtained every 6 months for 3 years and yearly thereafter.

In this review the results of all clinical trials have been updated as of December 1, 1983. In comparing disease-free and overall survival rates in the treatment arms at the completion of each study, the test of Mantel and Haenszel was used. Estimates of the survival curves were computed using the method of Kaplan and Meier. In comparing proportions in two groups, we have used the Fisher exact test.

Pilot Nonrandomized Trial of Adjuvant Chemotherapy Conducted Between May 1975 and June 1977

Between May 1975 and June 1977, pilot trials were initiated in the Surgery Branch of the National Cancer Institute evaluating the efficacy of adjuvant chemotherapy (with the regimen shown previously) in patients with soft tissue sarcomas of the extremities and the head, neck, and trunk. Twenty-six patients with high-grade extremity lesions were compared to 46 comparable historical controls from our institution who met eligibility criteria for the current protocol but who did not receive chemotherapy. Current analysis of these data with minimum follow-up in this trial of 6 years is shown in figures 1 and 2. The relapse-free, 5-year survival rate was 68% in chemotherapy protocol patients compared to 40% in historical control patients (P_1 = 0.004). The overall 5-year survival rate was 84% in patients treated with chemotherapy compared to 50% in historical controls (P_1 = 0.001).

Similarly, patients with head and neck and truncal lesions, including patients with retroperitoneal sarcomas, were evaluated compared to historical controls. Twenty-three protocol patients were compared to 20 historical controls. As shown in figures 3 and 4, there was no improvement in disease-free or overall survival in patients randomized to receive chemotherapy compared to those patients in historical controls (P_2 = 0.91 and 0.24, re-

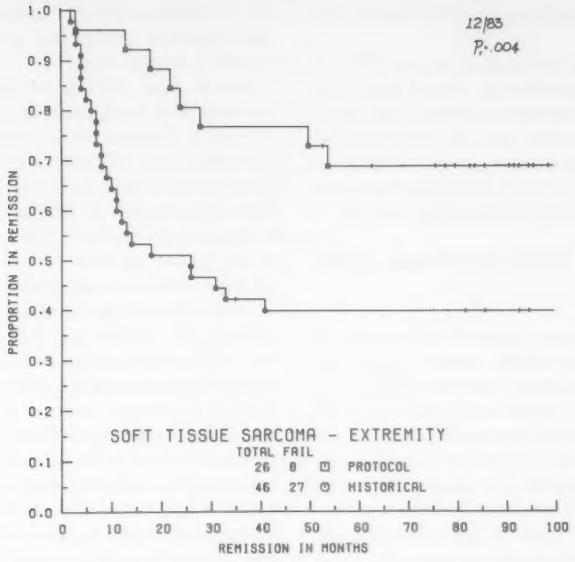

FIGURE 1.—Actuarial analysis of the continuous disease-free survival of patients with high-grade soft tissue sarcomas of the extremities treated in a pilot protocol between 1975 and 1977. Patients receiving adjuvant chemotherapy exhibited improved disease-free survival compared to historically controlled patients who did not receive chemotherapy.

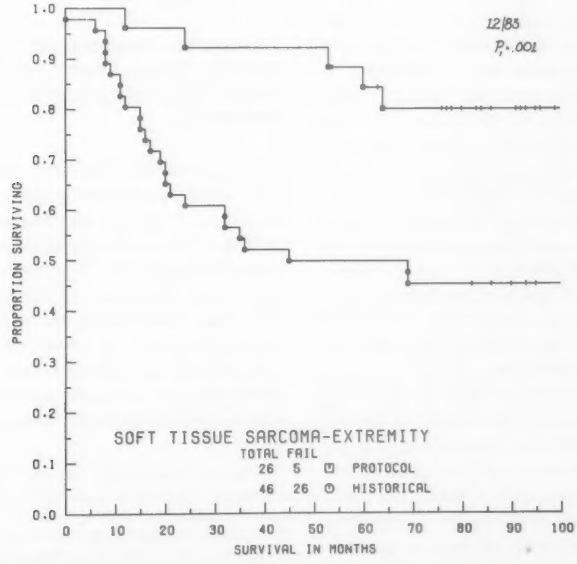

FIGURE 2.—Actuarial analysis of survival for the same patients shown in fig 1. Patients receiving chemotherapy exhibited prolonged survival compared to comparable historical controls.

spectively) in our current analyses (6-year minimum follow-up).

When this pilot trial was terminated in June 1977, it appeared that adjuvant chemotherapy was of benefit in patient groups with both extremity lesions and head, neck, and trunk lesions. Over the years, the difference between the groups has disappeared in patients with head, neck, and trunk lesions (figs 3 and 4) but has been sustained in patients with extremity lesions (figs 1 and 2).

Prospective Randomized Trial of the Efficacy of Adjuvant Chemotherapy

Early in our studies it became apparent that the use of doxorubicin and cyclophosphamide caused significant toxicity in these adjuvant patients. Cardiomyopathy due to doxorubicin was the most significant problem (15,16). About 10% of patients developed congestive heart failure due to this regimen and while most could be readily controlled with diuretics and digoxin, intractable congestive heart failure developed in two patients. In addition, it appeared that approximately 50% of asymptomatic patients had decreased left ventricular injection fractions as measured by ECG-gated radionuclide cineangiography when maximum doses (550 mg/m^2) of doxorubicin were given.

We thus initiated prospective randomized trials in patients with extremity and head, neck, and truncal high-grade sarcomas evaluating the efficacy of the adjuvant chemotherapy regimen detailed previously. Between June 1977 and July 1981, 65 patients with soft tissue sarcomas of the extremities were randomized into a treatment protocol in which local control was achieved either by amputation or by limb-sparing surgery plus radiation therapy. Patients were then randomized to either receive or not receive adjuvant chemotherapy. Median follow-up in this trial is 4½ years. The site, histology, grade, age, type of surgery, and final resection margins of the 65 patients entered in this trial are shown in table 1. Results are presented in figures 5 and 6. There was a substantial improvement in continuous disease-free and overall survival in patients randomized to receive chemotherapy ($P_1 = 0.008$ and $P_1 = 0.01$, respectively). The 5-year actuarial disease-free survival rate was 71% for patients receiving chemotherapy compared to 46% for those not receiving chemotherapy and the overall survival rate was 86% for patients receiving chemotherapy compared to 51% for those not receiving chemotherapy. This improvement in overall and disease-free survival was also seen in the subpopulation of patients with extremity lesions who had limb-sparing surgery (figs 7 and 8) ($P_1 = 0.03$ and $P_1 = 0.06$, respectively). Patients randomized to receive adjuvant chemotherapy also had a decreased incidence of local recurrence. Analyses of all patients randomized in the trial (fig 9) and of only those patients undergoing limb-sparing surgery (fig 10) demonstrated an improvement in local control in those patients randomized to receive chemotherapy ($P_1 = 0.01$ in both groups).

Also in June 1977, a prospective randomized trial of patients with head, neck, and truncal lesions was begun in which patients either received or did not receive chemotherapy. All patients in these groups received wide local excision plus radiation therapy in an attempt to achieve local control. This protocol has in fact continued to the present. Median follow-up in this trial is 35 months at the time of analysis. As is shown in figures 11 and 12, no improvement in either the disease-free or overall survival was seen in patients randomized to receive chemotherapy ($P_2 = 0.94$ and 0.42, respectively). Because of the small number of patients at these sites it is difficult to perform meaningful analyses of patient subpopulations. Preliminary analyses of patient subgroups, however, have shown no evidence of benefit due to adjuvant chemotherapy in patients with retroperitoneal sarcomas[4] or in patients with sarcomas in the head and neck or the breast.[5] However, in that small subgroup of patients with lesions on the trunk wall a substantial increase in disease-free survival is seen in preliminary analyses for those patients randomized to receive adjuvant chemotherapy. It should be emphasized, however, that patient numbers are small and accrual in these latter protocols is continuing. Because of the toxicity of the combined treatment with adjuvant chemotherapy and radiation therapy in patients with retroperitoneal sarcomas and the lack of benefit seen to the present time, adjuvant chemotherapy has, however, been discontinued in patients with retroperitoneal sarcomas.

Prospective Randomized Trial of Standard Chemotherapy Versus Short-Course Chemotherapy in Patients With High-Grade Soft Tissue Sarcomas of the Extremities

Because of the effectiveness of adjuvant chemotherapy that was demonstrated in patients with extremity lesions (figs 1, 2, 5, and 6), the prospective randomized trial of adjuvant chemotherapy in patients with high-grade soft tissue sarcomas of the extremities was discontinued in July 1981. Because of the cardiomyopathy seen with high-dose doxorubicin we then initiated a prospective randomized trial in which patients were prospectively randomized to receive either the standard adjuvant chemotherapy regimen previously shown or a shorter course of chemotherapy. This short-course chemotherapy

[4]Glenn J, Sindelar WF, Kinsella T, et al. Results of multi-modality therapy of resectable soft-tissue sarcomas of the retroperitoneum. Manuscript submitted to *Annals of Surgery*.

[5]Glenn J, Sindelar WF, Kinsella T, et al. A randomized prospective trial of adjuvant chemotherapy in adults with soft-tissue sarcomas of the head and neck, breast and trunk. Manuscript submitted to *Journal of Clinical Oncology*.

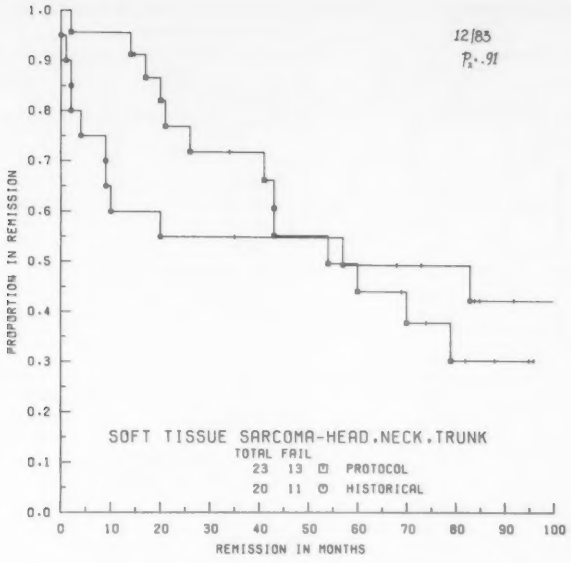

FIGURE 3.—Actuarial analysis of the continuous disease-free survival of patients with high-grade soft tissue sarcomas of the head, neck, and trunk treated in a pilot protocol between 1975 and 1977. Patients receiving adjuvant chemotherapy exhibited similar disease-free survival compared to historically controlled patients who did not receive chemotherapy.

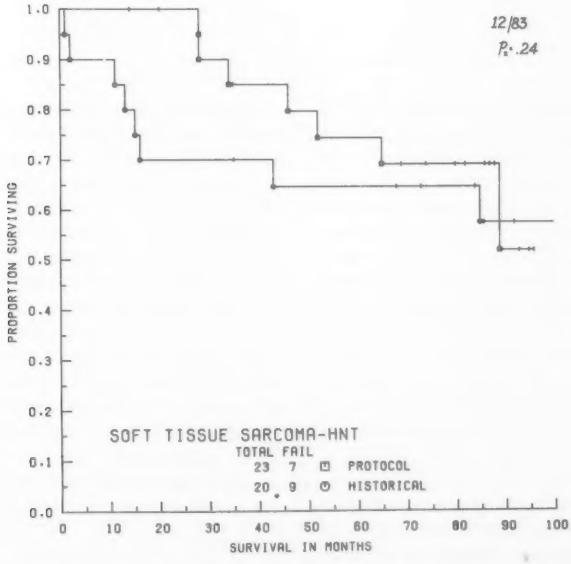

FIGURE 4.—Actuarial analysis of survival for the same patients with high-grade soft tissue sarcomas of the head, neck, and trunk shown in fig 3. Patients receiving chemotherapy exhibited similar survival compared to comparable historical controls.

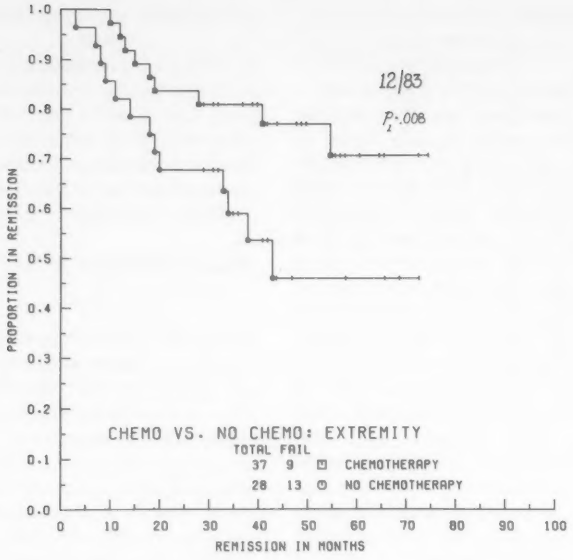

FIGURE 5.—Actuarial analysis of continuous disease-free survival in patients with high-grade soft tissue sarcomas of the extremities prospectively randomized to either receive chemotherapy or not receive chemotherapy. The chemotherapy regimen consisted of doxorubicin, cyclophosphamide, and high-dose methotrexate as described in the text. Patients randomized to receive adjuvant chemotherapy exhibited an improved continuous disease-free survival compared to patients not receiving chemotherapy.

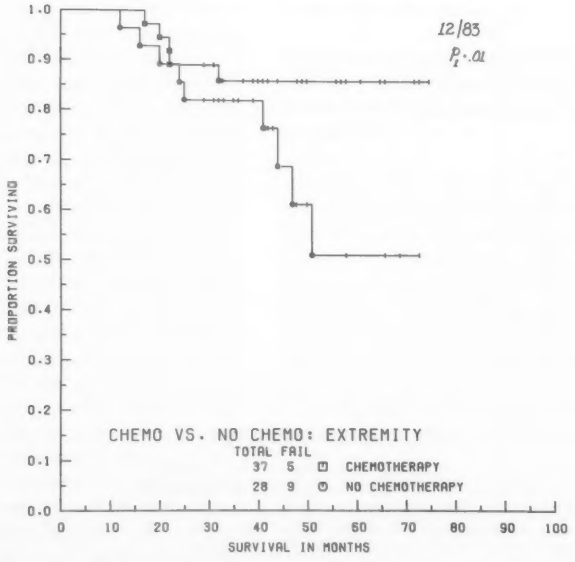

FIGURE 6.—Actuarial analysis of overall survival of the same patients shown in fig 5. Patients randomized to receive chemotherapy exhibited an improved survival.

TABLE 1.—Surgery Branch, National Cancer Institute randomized trial of the efficacy of adjuvant chemotherapy in patients with soft tissue sarcomas of the extremity

	No. of patients (%)		
	Chemotherapy	No chemotherapy	Total
Total	37	28	65
Site			
Arm	5 (13)	5 (18)	10 (15)
Forearm and hand	7 (19)	3 (11)	10 (15)
Thigh	7 (46)	16 (57)	33 (51)
Leg and foot	8 (22)	4 (14)	12 (19)
Histology			
Fibrosarcoma	1 (3)	4 (15)	5 (8)
Malignant fibrous histiocytoma	8 (22)	9 (33)	18 (27)
Liposarcoma	6 (16)	6 (21)	11 (17)
Leiomyosarcoma	2 (5)	2 (7)	4 (6)
Rhabdomyosarcoma	2 (5)	0 (0)	2 (3)
Synovial cell sarcoma	12 (33)	4 (14)	16 (24)
Neurofibrosarcoma	3 (8)	2 (7)	5 (8)
Unclassified	3 (8)	1 (3)	5 (7)
Grade			
1	0 (0)	0 (0)	0 (0)
2	6 (16)	8 (29)	14 (22)
3	31 (84)	20 (71)	51 (78)
Age (yrs)			
0–20	4 (11)	5 (18)	9 (14)
21–40	17 (46)	10 (36)	27 (41)
41–60	15 (41)	9 (32)	24 (36)
> 60	1 (2)	4 (14)	5 (8)
Surgery			
Amputation	16 (43)	11 (39)	27 (42)
Limb-sparing	21 (57)	17 (61)	38 (58)
Final resection margin			
Negative	33 (89)	26 (93)	59 (90)
Positive	3 (8)	0 (0)	3 (5)
Unknown	1 (3)	2 (7)	3 (5)

consisted of five doses of doxorubicin and cyclophosphamide given on Day 1 of a 28-day treatment cycle. Doxorubicin was given at a dose of 70 mg/m^2 and cyclophosphamide was given at a dose of 700 mg/m^2. After five doses all chemotherapy was discontinued. High-dose methotrexate was not administered. As of December 1, 1983, 79 patients had been randomized into this treatment trial. Thirty-five patients were randomized to receive standard chemotherapy and 44 were randomized to receive the pilot chemotherapy. Results as of December 1983 are presented in figures 13 and 14. As of the present time there is a trend towards improved disease-free and overall survival in patients randomized to receive the standard adjuvant chemotherapy (P_2 = 0.18 and 0.11, respectively), although follow-up is far too short to draw any definitive conclusions about this trial at this time.

General Conclusions

It thus appears, on the basis of our pilot trial and our prospective randomized trial, that adjuvant chemotherapy is of benefit in prolonging disease-free and overall survival in patients with high-grade soft tissue sarcomas of the extremities. We have not demonstrated a benefit of adjuvant chemotherapy in patients with high-grade soft tissue sarcomas of the head and neck, breast, and retroperitoneum, although preliminary analyses do reveal an improvement due to chemotherapy in patients with trunk wall lesions. Because of small patient numbers all trials in patients with head and neck, breast, and trunk wall lesions randomized to receive or not receive chemotherapy are continuing. We have, however, discontinued our trial of the role of adjuvant chemotherapy in patients with retroperitoneal sarcomas because of the toxicity of this combination regimen.

There is toxicity associated with the administration of doxorubicin and cyclophosphamide in the adjuvant setting. Most patients experienced nausea and vomiting for 12–24 hours following the drugs. Sterility was common when chemotherapy was administered in conjunction with radiation therapy near the testes or ovaries (17,18). The major toxic effect of this regimen, however, was

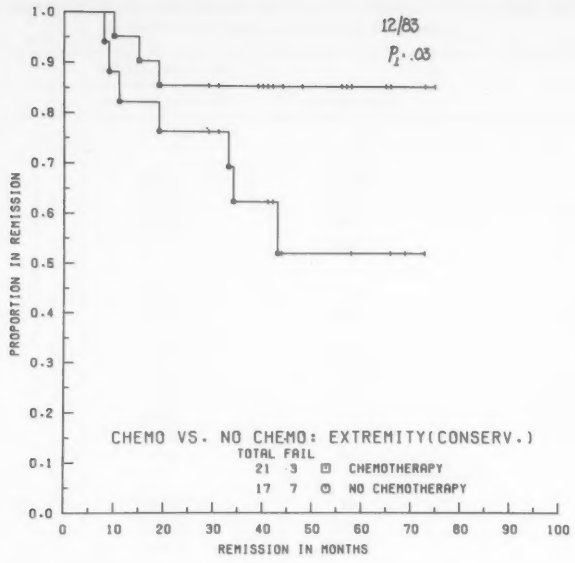

FIGURE 7.—Actuarial analysis of continuous disease-free survival of a subpopulation of patients shown in fig 5 who received limb-sparing surgery plus radiation therapy. Adjuvant chemotherapy was of benefit in prolonging disease-free survival in this patient subpopulation compared to similar patients randomized to receive no chemotherapy.

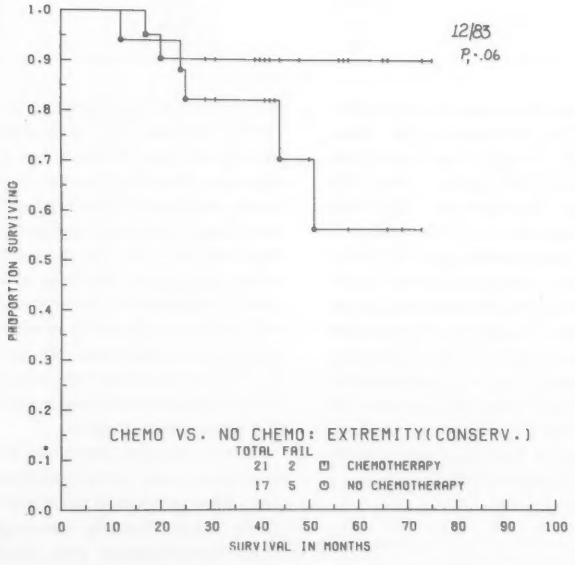

FIGURE 8.—Actuarial analysis of overall survival for the same patients shown in fig 7. Survival was improved in patients randomized to receive chemotherapy compared to those not receiving chemotherapy.

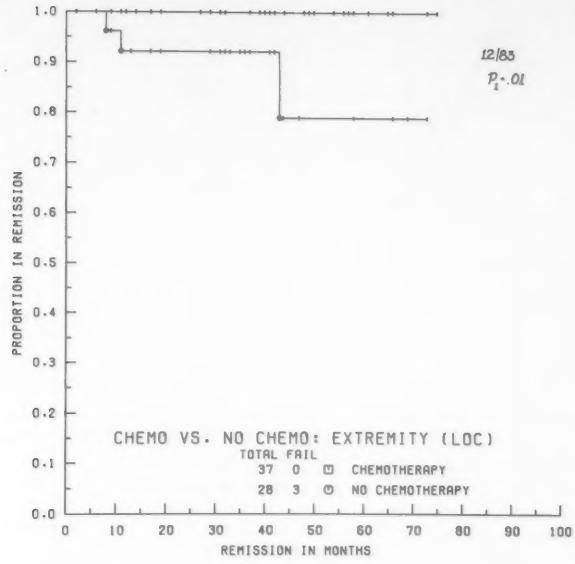

FIGURE 9.—Actuarial analysis of time to local recurrence in patients with high-grade soft tissue sarcomas of the extremities randomized prospectively to either receive or not receive chemotherapy. The patients receiving chemotherapy had a lower incidence of local recurrence than patients not receiving chemotherapy.

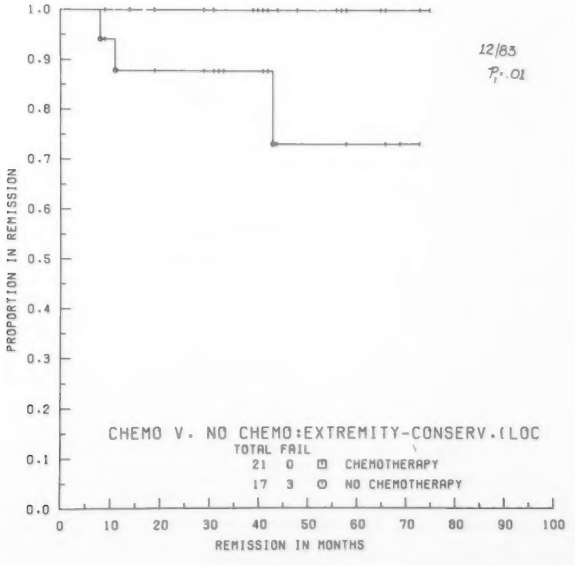

FIGURE 10.—Actuarial analysis of time to local recurrence in patients with high-grade soft tissue sarcomas of the extremities who underwent limb-sparing excision plus radiation therapy in an attempt to achieve local control. Patients randomized prospectively to receive chemotherapy had a lower incidence of local recurrence than patients randomized to receive no chemotherapy.

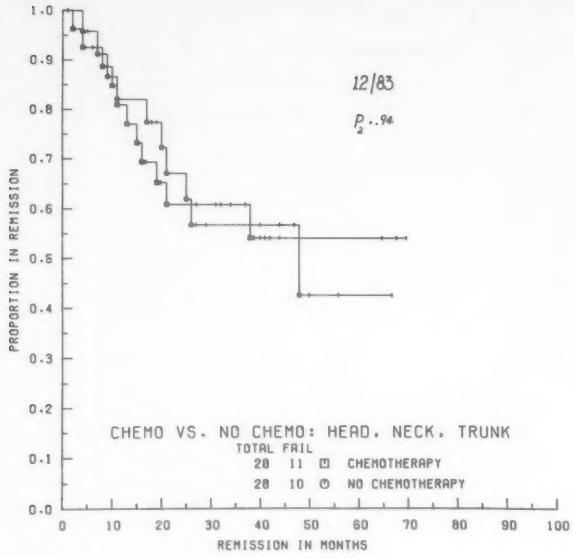

FIGURE 11.—Actuarial analysis of continuous disease-free survival of patients with head, neck, and truncal high-grade soft tissue sarcomas randomized to receive or not receive chemotherapy. No benefit of adjuvant chemotherapy was seen.

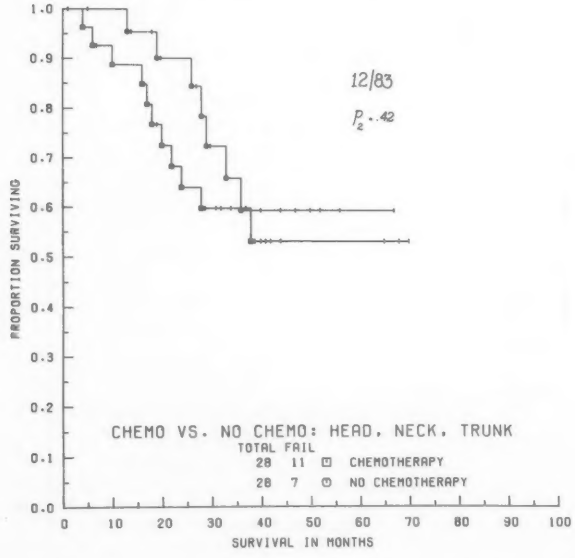

FIGURE 12.—Actuarial analysis of overall survival for the same patients shown in fig 11. No benefit in survival was seen in this patient population randomized to receive chemotherapy.

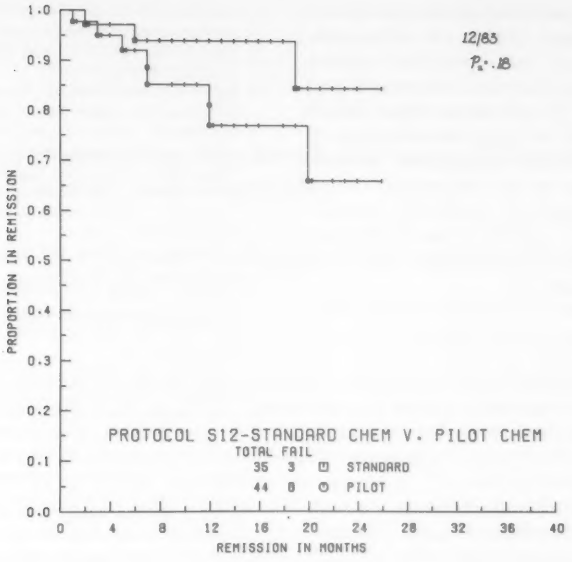

FIGURE 13.—Actuarial analysis of patients with high-grade soft tissue sarcomas of the extremities randomized to receive either our standard chemotherapy regimen (in text) or a shorter chemotherapy regimen consisting of only 5 doses of doxorubicin and cyclophosphamide. Follow-up is too short for definitive analysis of these data.

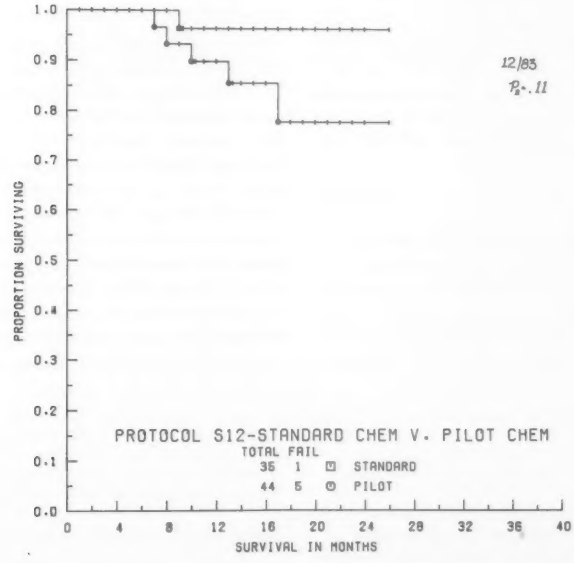

FIGURE 14.—Actuarial analysis of overall survival for the same patients shown in fig 13.

cardiomyopathy. We have performed extensive studies in these patients largely using ECG-gated radionuclide cineangiography to evaluate left ventricular ejection fractions. In 101 patients who received between 430 and 600 mg/m^2 of doxorubicin, 14 patients developed clinical cardiomyopathy manifested by signs and symptoms of congestive heart failure. Of 61 asymptomatic patients who underwent evaluation of left ventricular ejection fractions, 52% had abnormal left ventricular ejection fractions either at rest or at exercise. Although these patients are currently asymptomatic, long-term effects of these cardiac changes are unknown.

Because of this toxicity we have initiated a prospective randomized trial evaluating a lower cumulative dose of doxorubicin and cyclophosphamide which would be expected, on the basis of previous studies, to result in no clinical congestive heart failure and a decrease in left ventricular ejection fraction in only approximately 10% of asymptomatic patients. This trial is ongoing and the results are not yet available.

It appears, however, that adjuvant chemotherapy with doxorubicin and cyclophosphamide does improve disease-free and overall survival in patients with high-grade soft tissue sarcomas. A substantial challenge exists in developing adjuvant chemotherapy regimens with similar effectiveness but with decreased toxic effects.

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Combination Chemotherapy for Non-Small Cell Lung Cancer With Doxorubicin and Mitomycin or Cisplatin and Etoposide[1]

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For the Swiss Group for Clinical Cancer Research

The combinations of doxorubicin (40 mg/m² iv every 3 weeks) and mitomycin (12 mg/m² iv every 6 weeks) or cisplatin (80 mg/m² iv every 3 weeks × 3, then every 6 weeks) and etoposide (80 mg/m² iv on Days 1–3 every 3 weeks × 3, then every 6 weeks) were evaluated in a randomized phase II trial in 77 patients with measurable or evaluable non-small cell lung cancer. No patient had been pretreated with prior chemotherapy. The overall response rate for the doxorubicin and mitomycin regimen was 11% and for the cisplatin and etoposide regimen was 23%. Responses lasted a median of 5.5 months. Median survival was 7.0 months for the doxorubicin and mitomycin regimen and 8.0 months for the cisplatin and etoposide regimen. One treatment-related death was observed after doxorubicin and mitomycin in a patient who had received radiation therapy immediately prior to study entry. Otherwise, the hematologic toxicity of both chemotherapy regimens was moderate, with only six patients (10%) having World Health Organization grade 3 toxicity and no patients having World Health Organization grade 4 toxicity. However, subjective tolerance (gastrointestinal upset) of the cisplatin and etoposide combination was poor. [Cancer Treat Rep 68:1079–1084, 1984]

In an attempt to develop a chemotherapeutic treatment of non-small cell lung cancer with group-wide activity, the Swiss Group for Clinical Cancer Research evaluated two new promising regimens. The combination of doxorubicin and mitomycin was studied because both drugs have single-agent activity against non-small cell lung cancer (1–4) and because this combination had been reported to produce a 36% response rate in a small series of patients with adenocarcinoma of the lung (5). The second combination of cisplatin and etoposide was chosen because cisplatin is active in the treatment of non-small cell lung cancer (6,7). Furthermore, evidence of a therapeutic synergism in murine tumors treated with the cisplatin and etoposide combination has been reported (8), and in our own pilot study this regimen produced a remission rate of 33% (9). Patient accrual started in September 1980 and 89 patients were entered in the study until December 1981. The results of this trial serve as a basis for this report.

PATIENTS, MATERIALS, AND METHODS

Patients

Patients with histologically proven, surgically incurable epidermoid carcinoma, adenocarcinoma, or large cell anaplastic carcinoma of the lung were eligible for this study. Measurable (two perpendicular diameters measurable) or evaluable (one diameter measurable) lesions were required and patients with either locally advanced or

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metastatic disease were accepted for the study. Patients with prior chemotherapy, irradiation to areas of measurable disease, brain metastases, performance status of 4 (World Health Organization [WHO] scale), or > 70 years of age were ineligible. Adequate pretreatment renal (creatinine ⩽ 1.30 mg/dl and creatinine clearance ⩾ 50 ml/minute), hepatic (bilirubin < 2.0 mg/dl and SGOT and SGPT < 3 × the upper limit of normal), marrow (wbc count ⩾ 3500/mm^3 and platelet count > 100,000/mm^3), and cardiac (no myocardial infarction during 6 months prior to study entry, no signs of congestive heart failure, and no arrhythmias) functions were required. All patients gave informed consent to participate in this clinical trial.

Study Design

Patients were stratified by histologic subtype, extent of disease, and performance status and were then randomized to one of the two treatment arms. Serial observations before and during treatment included history and physical examination, measurement of indicator lesions and performance status, cbc, platelet count, and blood chemistries. A bone scan was performed in all patients prior to entrance in the study and was repeated as indicated. Screening for brain and liver metastases was not performed routinely, but patients with symptoms and signs suggesting CNS and/or liver involvement were evaluated by computerized tomographic scan or sonography. The radiographic evaluation included posteroanterior and lateral chest films and x-rays of areas positive on bone scan or suspected of having metastatic disease from the history and physical examination.

The response to treatment was assessed for the first time after 6 weeks according to WHO response criteria (10). Bronchoscopies were not repeated to confirm the completeness of a response. Death before the initial response assessment at 6 weeks was considered as early death. Any death to which drug toxicity was thought to have made a major contribution was considered a toxic death. The duration of response was calculated from the first day of treatment until the documentation of disease progression. Survival was dated from the start of treatment.

Treatments

The therapeutic regimen of doxorubicin and mitomycin consisted of doxorubicin at a dose of 40 mg/m^2 iv on Days 1 and 22 and mitomycin at a dose of 12 mg/m^2 iv on Day 1, repeated every 6 weeks. Patients treated with cisplatin and etoposide received cisplatin at a dose of 80 mg/m^2 iv on Day 1 and etoposide at a dose of 80 mg/m^2 iv on Days 1–3 every 3 weeks for three cycles, then every 6 weeks.

Cisplatin was infused in 250 ml of normal saline over 1 hour after prehydration with 1000 ml of 5% dextrose in one-half normal saline over 2 hours. A large diuresis was maintained after cisplatin administration through adequate fluid replacement and furosemide, if diuresis did not exceed 100 ml/hour.

Planned drug administration was delayed for wbc counts ⩽ 3500/mm^3 or platelet counts ⩽ 100,000/mm^3, for a maximum of 2 weeks. Thereafter, the doses were reduced by 50%, for a wbc count of 2500–3500/mm^3 and a platelet count of 70,000–100,000/mm^3. If the wbc count nadir was ⩽ 1000/mm^3 or the platelet count nadir was ⩽ 50,000/mm^3, the doses of cisplatin, etoposide, and doxorubicin were reduced by 25% and the dose of mitomycin was reduced by 50% at the next treatment.

Patients progressing under one treatment regimen were crossed over, if possible, to the other treatment arm.

RESULTS

Patient Characteristics

Of 89 patients entered in the study, 77 were eligible for analysis. Twelve patients were considered ineligible for the following reasons: four patients were cancelled prior to therapy, three had no measurable disease, and one each was excluded for age > 70 years, small cell histology, prior chemotherapy, prior radiation therapy to the area of measurable disease, and CNS metastases. The patient characteristics of the 77 evaluable patients are shown in table 1 according to the treatment regimens. Eight patients were classified as early deaths, six on the doxorubicin and mitomycin arm and two on the cisplatin and etoposide arm. Of these patients, three probably died due to rapidly progressive disease on Days 15, 22, and 33, whereas five died of other causes (aortic dissection under anticoagulation on Day 18, sudden death at home on Day 21, gastrointestinal hemorrhage with normal platelet count on Day 9, cerebrovascular accident on Day 8, and pulmonary embolus on Day 6). Two patients in the cisplatin and etoposide arm refused treatment after the first cycle. One patient was considered to have early toxic death. This patient was irradiated 11 days prior to study entry to a painful thoracic vertebral body with 800 rads in a single fraction followed by three fractions of 400 rads daily. Seven days after completion of radiation therapy doxorubicin and mitomycin were administered. The patient developed granulocytopenia with gram-negative septicemia and died 30 days after his first cytostatic treatment.

Response and Survival

The observed responses and their distribution according to selected prognostic factors are shown in table 2. In the doxorubicin and mitomycin arm we observed one complete remission (CR) and three partial remissions

TABLE 1.—Patient characteristics according to treatment regimen*

Characteristic	Regimen	
	Doxorubicin/mitomycin	Cisplatin/etoposide
Eligible patients	37	40
Median age in yrs (range)	58 (43-70)	58 (44-68)
Male:female	34:3	37:3
Performance status		
0-1	28	30
2-3	9	10
Weight loss		
< 5%	15	21
> 5%	17	15
Unknown	5	4
Histology		
Epidermoid carcinoma	20	21
Adenocarcinoma	8	10
Large cell carcinoma	9	9
Stage		
Limited disease	19	16
Extensive disease	18	24
Prior treatment		
None	28	29
Surgery	6	6
Radiotherapy	1	3
Surgery + radiotherapy	2	2

* Unless otherwise specified, values = No. of patients.

(PRs), for an overall response rate of 11% (one CR + three PRs among 37 patients) (predicted true response rate 4%-25% at the 95% confidence limit). The patient with a CR was treated for locoregional epidermoid carcinoma. He had experienced prior loss of > 5% of body weight and had a performance status of 1. The remission lasted for 10.5 months and the patient is currently alive 20.5 months after study entry. The three PRs were achieved in one extensive epidermoid and in two limited large cell carcinomas. The remissions lasted for 5, 8.5, and 9 months, respectively. In the cisplatin and etoposide arm one CR and eight PRs were achieved, for an overall remission rate of 23% (one CR + eight PRs among 40 patients) (predicted true response rate, 11%-38% at the 95% confidence limit). One patient with disseminated epidermoid lung cancer with prior loss of < 5% of body weight and a performance status of 1 achieved a CR lasting for 3 months. The patient then developed brain metastases and the treatment was interrupted. The PRs were achieved in six patients with epidermoid carcinoma and in one patient each with adenocarcinoma and large cell carcinoma. Five of these patients had limited and three had extensive disease. The cisplatin and etoposide regimen exhibited greater activity in epidermoid carcinoma (one CR + six PRs among 21 patients, 33%) than in the other histological subtypes (two PRs among 19 patients, 11%), but the difference did not reach statistical significance ($P > 0.05$). The eight PRs achieved with the cisplatin and etoposide regimen lasted a median of 5.0 months (range, 3.5-6.0).

Six patients receiving the doxorubicin and mitomycin arm (no change, four patients; progressive disease, two) were crossed over to the treatment with cisplatin and etoposide. Four patients progressed under the second-line cytostatic treatment and two patients had stable disease for 3 and 13 months, respectively. Fourteen patients with an initial treatment of cisplatin and etoposide (PR, four patients; no change, five; and progressive disease, five) were subsequently treated with doxorubicin and mitomycin. Five patients had stable disease for 2+, 3, 3+, 3.5, and 6 months, respectively; nine had further progression of their disease.

Median survival was 7.0 months for the patients with the doxorubicin and mitomycin regimen and 8.0 months for the patients treated with the cisplatin and etoposide combination. Survival according to the remission status and treatment is shown in table 2.

Toxicity

The major toxic effect seen was myelosuppression (table 3). The doxorubicin and mitomycin regimen induced only modest hematologic toxicity in slightly more than one-half of the patients. However, as mentioned above, this regimen induced one early toxic death in a patient who had received radiation therapy to a small area of the

TABLE 2.—Survival and responses according to treatment regimen and prognostic factors*

	Regimen									
	Doxorubicin/mitomycin					Cisplatin/etoposide				
	CR	PR	NC	P	NE	CR	PR	NC	P	NE
Eligible patients	1	3	21	5	7	1	8	16	11	4
Performance status										
0–1	1	3	18	4	2	1	6	12	9	2
2–3	0	0	3	1	5	0	2	4	2	2
Weight loss										
< 5%	0	3	8	3	1	1	3	8	6	3
> 5%	1	0	12	0	4	0	5	5	4	1
Unknown	0	0	1	2	2	0	0	3	1	0
Histology										
Epidermoid carcinoma	1	1	13	2	3	1	6	5	8	1
Adenocarcinoma	0	0	4	2	2	0	1	5	2	2
Large cell carcinoma	0	2	4	1	2	0	1	6	1	1
Stage										
Limited disease	1	2	14	1	1	0	5	8	2	1
Extensive disease	0	1	7	4	6	1	3	8	9	3
Prior treatment										
None	1	3	17	3	4	0	6	14	8	1
Surgery	0	0	4	1	1	0	1	1	2	2
Radiotherapy	0	0	0	0	1	0	1	0	1	1
Surgery + radiotherapy	0	0	0	1	1	1	0	1	0	0
Median survival (mos)†	20.5	10.5	7.0	2.5	0.6	7.0	10.0	9.5	3.5	2.0

* Unless otherwise specified, values = No. of patients. NC = no change; P = progressive disease; and NE = not evaluable for antitumor activity.

†Overall median survivals were 7.0 mos (range, 0.3–21.0+) for the doxorubicin + mitomycin regimen and 8.0 mos (range, 0.2–18.0+) for the cisplatin + etoposide regimen.

thoracic spine shortly before study entry. Hematological toxicity was more pronounced with the cisplatin and etoposide regimen. Gastrointestinal toxicity was almost universal in the cisplatin and etoposide arm, with one-third of the patients experiencing severe nausea and vomiting, whereas gastrointestinal upset was less pronounced with the doxorubicin and mitomycin regimen. Four patients receiving the doxorubicin and mitomycin arm showed a transient increase of the serum creatinine to a maximum of 1.79 mg/dl (range, 1.52–1.79), with one patient exhibiting a syndrome of microangiopathic hemolytic anemia (this patient died due to disease progression). Nine patients receiving the cisplatin and etoposide arm had a reversible increase in the serum creatinine to a maximum of 1.75 mg/dl (range, 1.43–1.75), and one patient developed hypercalcemia with a concomitant increase in the serum creatinine to 1.93 mg/dl. One patient on the cisplatin and etoposide arm developed a peripheral sensory polyneuropathy and one patient developed a transient ageusia. The degree of stomatitis was mild to moderate in all five patients shown in table 3. Three patients on the doxorubicin and mitomycin arm developed evidence of cardiac toxicity. One patient developed atrial fibrillation after a cumulative dose of 200 mg/m^2 of doxorubicin (with no prior radiation therapy). He was successfully treated with digoxin and removed from the study. Two patients developed congestive heart failure after 200 and 400 mg/m^2 of doxorubicin, respectively. In both patients symptoms subsided promptly with digoxin.

DISCUSSION

The results of this trial illustrate once more the well-known fact that there are large discrepancies between results generated by single institutions and cooperative groups. The doxorubicin and mitomycin regimen was reported in a small single-institution trial to produce a promising response rate of 36% in adenocarcinoma of the lung (5). In the present study we observed an overall response rate of 11% in 37 patients, none of them having received prior chemotherapy. One might argue that almost one-half of our patients did not exhibit hematologic toxicity and that therefore the doxorubicin and mitomycin regimen was underdosed. However, our results are consistent with the findings of Coates et al (11), who reported an overall response rate of 13% in 30 patients with a similar treatment regimen, but at higher doses (doxorubicin, 50 mg/m^2 and mitomycin, 20 mg/m^2). The response rate of the doxorubicin and mitomycin combination is similar to the rate observed with either agent alone.

The cisplatin and etoposide combination had shown considerable activity in a pilot study performed by our group (9). Furthermore, preliminary data of the Belgian

TABLE 3.—Toxic effects according to treatment regimen*

	Regimen	
	Doxorubicin/mitomycin	Cisplatin/etoposide
Evaluable for toxicity	29	32
Hemoglobin nadir in g/dl (range)	10.9 (4.8-13.8)	11.5 (7.5-14.8)
Day of nadir (range)	55 (4-236)	54 (10-220)
Leukocyte count nadir (range), $\times 10^3$ cells/ml	3.8 (1.2-8.6)	2.7 (1.3-9.4)
≥ 4	13	7
3-3.99	9	8
2-2.99	5	13
1-1.99	2	4
< 1	0	0
Day of nadir (range)	74 (8-282)	35 (15-107)
Platelet count nadir (range), $\times 10^3$ cells/ml	152 (45-300)	148 (62-354)
≥ 100	22	26
50-99	6	6
20-49	1	0
Day of nadir (range)	58 (7-239)	41 (7-239)
Nausea/vomiting		
None	5	1
Moderate (WHO grades 1 and 2)	21	21
Severe (WHO grades 3 and 4)	3	10
Renal toxic effects (serum creatinine ≥ 1.4 mg/dl)	4	10
Alopecia		
None	3	4
Moderate	10	18
Severe	16	10
Neurological side effects	0	2
Stomatitis	3	2
Diarrhea	0	1
Cardiac toxicity	3	0

* Unless otherwise specified, values = No. of patients.

European Organization for Research on Treatment of Cancer Lung Cancer Working Party indicated that with a similar combination a 45% response rate with 10% CRs was achieved in 30 evaluable patients (12). In our group-wide trial the response rate dropped considerably, with a response rate of 23% in a group of 40 patients with favorable prognostic factors: none of them had received prior chemotherapy, most were ambulatory, one-half had experienced only minimal prior weight loss, and almost one-half had disease confined to the chest. However, we feel that this particular combination has activity comparable to other treatment regimens used in non-small cell lung cancer. The cumulative response rate from data available in the literature is 32% in 288 evaluable patients (9,13-17, present trial). Whether the cisplatin and etoposide combination is superior to either cisplatin alone or to other currently used chemotherapy regimens warrants further randomized studies such as are currently ongoing within the Eastern Cooperative Oncology Group. As with other cisplatin-containing regimens, subjective tolerance worsened with an increasing number of treatment courses. Prolongation of the treatment intervals after the third course did not improve gastrointestinal tolerance compared to our previous experience (9). Four of 40 patients refused further treatment with cisplatin and etoposide after the first or one of the following cycles due to nausea and vomiting.

In conclusion, our results suggest that the cisplatin and etoposide combination has activity against non-small cell lung cancer. However, in view of the marked gastrointestinal side effects and the rather short remission duration, we feel that this combination cannot be recommended as standard chemotherapy for non-small cell lung cancer and that effective cytostatic treatment of this disease remains an elusive goal.

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Phase I Clinical Investigation of Homoharringtonine[1,2]

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Homoharringtonine is a cephalotaxine ester derived from *Cephalotaxus harringtonia*, which is a Chinese evergreen tree. A limited clinical evaluation of this drug in China revealed antileukemic activity, which prompted clinical trials in the United States. We have treated 43 patients with a variety of refractory malignancies using a daily iv treatment for 5 days at 3–4-week intervals. The starting dose of homoharringtonine was 0.2 mg/m²/day and it was escalated to a maximum of 8 mg/m²/day. The dose-limiting toxic effect was hypotension, which was generally mild with daily dose levels of 3–4.5 mg/m²/day and required no specific treatment besides iv fluid supplements in some patients. Hypotension became increasingly severe at the higher dose levels and resulted in cardiovascular collapse in four of 16 patients treated with dose levels of 5–6 mg/m²/day. A moderately severe degree of myelosuppression was observed with homoharringtonine doses of ≥ 3 mg/m². Myelosuppression was clearly related to the extent of prior treatment and was minimal in patients who had not received extensive prior treatment. Gastrointestinal toxic effects of nausea, vomiting, and diarrhea were observed in approximately two-thirds of the patients but these side effects were generally mild and self-limited. Drug-related fever and alopecia were also observed in some patients. No major responses were observed, although three patients with solid tumors evidenced minor responses and three of five patients with acute leukemia showed some degree of antileukemic activity. For phase II studies of homoharringtonine in solid tumors, a daily dose of 3 mg/m² for 5 days in patients with extensive prior treatment and 4 mg/m²/day for 5 days in patients with good bone marrow reserve will be utilized. The daily dose must not exceed 4 mg/m² to avoid serious hypotension; further dose escalations should be accomplished by extending the number of days of treatment beyond 5 days. [Cancer Treat Rep 68:1085–1091, 1984]

Homoharringtonine is one of a group of cephalotaxine esters originally isolated by Powell and co-workers (1) in 1969. The source of this drug and other related alkaloids such as harringtonine is an evergreen tree, *Cephalotaxus harringtonia*, which is widely distributed throughout China. Because plants yield greater quantities of homoharringtonine and the preclinical activities of homoharringtonine and harringtonine are roughly comparable, homoharringtonine (fig 1) has been chosen for further evaluation, although both agents have similar biochemical effects. In determining the mechanism of action, evidence indicates that harringtonine and probably homoharringtonine inhibit biosynthesis of protein, DNA, and RNA via inhibition of chain initiation. Preclinical screening indicated good antitumor activity in murine tumors P388 and colon 38, moderate activity against CD8F1 mammary carcinoma and L1210 leukemia, and slight activity against B16 melanoma (2). Homoharringtonine was not active against Lewis lung carcinoma, human colon, lung, or mammary tumor xenografts. The antitumor activity of homoharringtonine against P388 was highly schedule-dependent, with daily treatment for 9 days being more effective than intermittent administration on Days 1, 5, and 9, which in turn was more effective than single-dose administration (2). Also, for any given schedule, administration of several doses throughout the

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day was more effective than administration of one large dose. Animal toxicology studies were performed in mice and beagle dogs. The LD10 of homoharringtonine in mice was 12.8 mg/m^2 with a single dose, compared to 5.82 mg/m^2 on a daily × 5-day schedule. The toxic effects of homoharringtonine in mice and dogs included anemia, leukopenia, thrombocytopenia, hypoplasia of the bone marrow, vomiting, and hemorrhagic diarrhea. There was also some evidence of cardiac toxicity manifested as sinus tachycardia, ventricular arrhythmias, and possible congestive heart failure. Histologic examination of the myocardium revealed focal degenerative changes in one dog given the LD10 dose. No such lesions were seen in mice and it is believed that such changes may have been related to or be secondary to hypovolemic shock. Mild biochemical changes indicative of liver toxicity, as well as renal toxicity, were also observed.

Several clinical trials with harringtonine and homoharringtonine have been published in the Chinese literature (3). In most instances, the drug used in the Chinese studies was a mixture of harringtonine and homoharringtonine and the patient population predominantly had acute leukemia. The drug was generally administered im or as a short iv infusion and the daily dosage ranged from 2 to 4 mg for 2-3-week periods. Complete remissions were achieved in 10%-15% of the patients with acute leukemia, mostly in previously untreated patients. Patients required two courses of chemotherapy and a cumulative dose of 100-150 mg/m^2 before achieving complete remission. The major toxic effects associated with the treatment were myelosuppression and cardiac rhythm disturbances. The maximum tolerated and therapeutically effective dose was thought to be 4 mg/day × 14 days. Cardiac abnormalities included sinus tachycardia in most patients and temporary ST-T wave changes in some patients. Premature ventricular contractions were also recorded in some patients. The drug caused nausea, vomiting, and stomatitis at the higher dose levels.

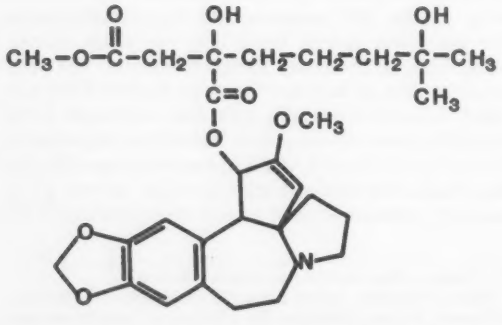

FIGURE 1.—Structure of homoharringtonine.

Homoharringtonine has also been reported to have been used in some patients in Australia, where it induced a complete remission in a patient with acute myeloblastic leukemia. The dosage was 0.3 mg/kg/day (12 mg/m^2) continuous infusion given over 24 hours for 5 days (2). This patient was apparently refractory to several different chemotherapeutic agents, indicating lack of cross-resistance with the other antileukemic drugs.

Based on these encouraging results, homoharringtonine was chosen for further evaluation in the United States. The drug used in the US trials is more highly purified and, accordingly, its equivalence to the Chinese preparations is uncertain. In this report, we describe the results of our phase I study of homoharringtonine using a daily × 5-day schedule.

PATIENTS AND METHODS

Patients with histologic proof of malignancy refractory to standard treatment modalities were eligible for this study. The study was initially limited to patients with solid tumors, but patients with hematologic malignancies were also treated after the maximum tolerated dose for the patients with solid tumors was defined. Presence of measurable lesions was not a prerequisite for patient entry. Patients were required to have an estimated life expectancy of at least 6 weeks and to have adequate bone marrow function defined as a peripheral absolute neutrophil count of $> 2000/mm^3$ and a platelet count of $> 100,000/mm^3$. Significant renal function abnormalities (serum creatinine $\geq$ 2.0 mg/100 ml) or liver dysfunction (bilirubin > 2 mg/100 ml) were considered contraindications to homoharringtonine treatment. Patients had to have been off all previous chemotherapy or radiotherapy for a period of 3-4 weeks and to have recovered completely from the toxic effects of previous treatment. Patients were made aware of the investigational nature of this study and signed informed consent to receive this investigational treatment.

Homoharringtonine was supplied by the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. It was made available in 5- and 10-mg vials in dry form. The contents of each vial of homoharringtonine were dissolved in dehydrated alcohol and sterile water and the resultant solution was stable for 24 hours. Homoharringtonine was administered as a daily iv infusion for 5 days, and the courses of chemotherapy were repeated at 3-4-week intervals depending on the patient's recovery from the toxic effects of the preceding course. The starting dose of homoharringtonine was 0.2 mg/m^2 × 5 days, which is $\frac{1}{10}$ of $\frac{1}{3}$ of the LD10 dose in mice. Each daily dose was dissolved in 200-500 ml of 5% dextrose solution or in normal saline and was infused over 1 hour. After a minimum of three patients were treated and followed up to 3 weeks, the homoharringtonine dose was escalated by

doubling the previous dose level until some evidence of biological effect was noted. The dose escalation rate was then reduced to 50% if the biological effects were minimal and to 25% if mild to moderate effects were clearly present. A minimum of three to four patients were treated at each of the dose levels until definite evidence of moderately severe toxicity was present and a minimum of six patients were planned to be treated at that dose level. Dose escalation in individual patients was allowed in the earlier part of the study, but once biological effects were clearly present, each patient was treated at the same dose level in subsequent courses of treatment. The courses of chemotherapy were repeated as long as there was no clear evidence of disease progression.

A majority of patients received homoharringtonine in the outpatient clinic. Because of the reported risk of cardiac toxicity, all patients had pretreatment ECGs and close monitoring of vital signs before, during, and at the end of infusion. Once hypotension was discovered to be the dose-limiting toxicity of homoharringtonine, five patients were admitted to the medical intensive care unit for cardiac monitoring and study of hemodynamic effects of the drug. An arterial line was placed to monitor the blood pressure changes at 1-hour intervals. A thermodilution Swan Ganz catheter was inserted to record the cardiac output before and immediately after drug infusion and at 6–8 hours after the infusion. Cbcs, differential counts, and platelet counts were checked one to two times every week, and SMA-12 determinations, which included tests of liver and renal function, were performed once every week. The tumor measurements were recorded before each course of homoharringtonine, and the response was assessed using the standard criteria. Patients were considered to have tumor progression if there was unequivocal increase by > 50% in the size of any measurable lesion or if new lesions developed.

RESULTS

Our phase I study of homoharringtonine was initiated in October 1981 and was completed by November 1982. The general characteristics of the 143 patients registered in the study are summarized in table 1. All patients had received extensive prior chemotherapy. The general performance status of patients was rather poor, with seven patients classified as grade 4. Eight patients were > 65 years of age. The histologic diagnoses of the patients treated with homoharringtonine are also shown in table 1. Most patients had solid tumors; however, five patients with acute leukemia were treated at the higher dose levels in the latter part of the study.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total treated	43
Median age in yrs (range): 49 (22–81)	
Performance status*	
1	13
2	12
3	11
4	7
Prior chemotherapy	43
Median No. of regimens (range): 3 (1–7)	
Prior radiotherapy	14
Tumor types	
Colon	10
Lymphoma	5
Acute leukemia	5
Melanoma	4
Sarcoma	3
Breast	3
Unknown primary	3
Lung	2
Ovary	2
Miscellaneous	6

* Eastern Cooperative Oncology Group criteria.

Dose Levels

A total of 43 patients received 89 courses of homoharringtonine at dose levels ranging from 0.2 to 8 mg/m^2 daily for 5 days (table 2). Seven patients failed to complete their first course of treatment, two because of early deaths and five because of serious toxicity following one or two doses of the 5-day course. The dose levels from 0.2 to 1.5 mg/m^2 were not associated with side effects. At the dose level of 3 mg/m^2, significant myelosuppression was observed in patients with extensive prior treatment but not in good-risk patients. Eleven patients were treated with 4 mg/m^2/day with variable effects on blood cell counts. The dose level of 5–5.5 mg/m^2 resulted in significant side effects in most patients with solid tumors. Five patients with acute leukemia were treated with a dose range of 5–6 mg/m^2, except for one patient who received 8 mg/m^2, which resulted in serious toxicity. Because hypotension was the limiting toxic effect with the dose levels of 5–6 mg/m^2, we subsequently elected to lower the daily dose to 4 mg/m^2 and extended the treatment to 6 days in patients who had no significant toxic effects with 4.0 mg/m^2 for 5 days. Five patients were treated with this dose level, none experienced severe hypotension, and the degree of myelosuppression was modest and quite acceptable.

Antitumor Activity

Among the patients with solid tumors, two experienced minor tumor regression lasting 6 and 8 weeks. One patient had metastatic breast cancer involving the bones, and a second had ovarian carcinoma with ascites. Among the patients with leukemia, there was complete clearance of circulating blasts in three of the five patients. One pa-

TABLE 2.—Dose escalation scheme and No. of patients treated at each dose level of homoharringtonine

Dose (mg/m^2 × 5 days)	No. of patients*	No. of courses		
		Initial	Subsequent	Total
0.2	3	3	0	3
0.4	5	2	3	5
0.8	6	2	6	8
1.5	6	2	4	6
3.0-3.5	14	11	11	22
4.0-4.5	16	13	10	23
5.0-5.5	9	6	7	13
6.0	7	3	6	9
8.0	1	1	0	1

* The total No. of patients in this table exceeds the No. of patients shown in table 1 because some patients were treated at > 1 dose level and were therefore counted multiple times.

tient also showed reduction in bone marrow leukemic infiltrate from 90% to 10% after three courses of therapy at 6 mg/m^2/day. The remaining two patients showed marked reduction in circulating blasts following a single dose of homoharringtonine. A patient with large cell lymphoma showed a transient shrinkage of the cervical lymph nodes by 50% but had no effect on bone marrow infiltrate.

Toxic Effects

Hypotension

Hypotension was the dose-limiting toxic effect of homoharringtonine. Both systolic and diastolic blood pressures decreased to a similar degree. Hypotension was first observed with a 3-3.5-mg/m^2 dose level (table 3). Four of 14 patients at this dose level developed mild hypotension, defined as a fall in systolic blood pressure of 20-30 mm Hg. At the dose level of 4-4.5 mg/m^2, six of 16 patients developed mild hypotension. One of these patients developed pulmonary edema following a relatively small infusion of iv fluids and the homoharringtonine had to be discontinued. The heart failure resolved completely with digoxin and lasix. Of note, this patient had previously received doxorubicin in a cumulative dose of 450 mg/m^2. Hypotension was more frequent and more severe at dose levels of 5-6 mg/m^2, affecting nine to 16 patients. Five patients became severely hypotensive and required vigorous supportive measures that included iv fluids for all patients and vasopressor agents for four. The highest dose of homoharringtonine used in this study was 8 mg/m^2 in one patient. Six hours following the first dose, the patient developed shock requiring vasopressors, but recovered after 72 hours of supportive therapy. Because no alternative therapy was available for his leukemia, he consented to receive a second course of homoharringtonine 10 days after the first course. Despite dose reduction to 6 mg/m^2 given as a slow infusion over 6 hours, the patient developed a fatal episode of cardiovascular collapse. During supportive measures with iv fluids and vasopressors for hypotension, three patients developed pulmonary edema; two of them improved with diuretics and digoxin therapy and the third patient died.

As shown in table 3, 20 of 47 patients (43%) treated with homoharringtonine in the dose range of 3-8 mg/m^2/day developed hypotension, which was mild in 14 patients and severe in six. All eight patients with elevated bilirubin and/or SGOT levels developed hypotension compared to 12 of 39 patients with normal liver function ($P < 0.01$). Hypotension was generally mild in patients treated with 3-4.5 mg/m^2 but was severe in one-

TABLE 3.—Hypotension associated with homoharringtonine*

Dose (mg/m^2)	No. of patients			
	Treated	Mild hypotension	Severe hypotension	Total mild + severe
0.2-1.5	20	0	0	0
3.0-3.5	14	4	0	4
4.0-4.5	16	6	0	6
5.0-5.5	9	2	3	5
6.0	7	2	2	4
8.0	1	0	1	1

* Mild hypotension = decrease of 20-30 mm Hg; severe hypotension = decrease of > 30 mm Hg, resulting in a systolic blood pressure of < 90 mm Hg.

third of the patients treated with dose levels of ≥ 5 mg/m^2. To unravel the etiology of hypotension, five patients receiving 4-6 mg/m^2 of homoharringtonine per day had monitoring of their blood pressure and other cardiovascular functions. All patients developed sinus tachycardia of 120-140/minute. Two patients developed ventricular ectopic beats and one developed first-degree atrioventricular block followed later by a right bundle branch block. Tachycardia was first observed 6-10 hours after administration of the first dose and generally coincided with the onset of hypotension. Hypotension, both systolic and diastolic, was observed in all patients in the dose range tested. Hypotension was accompanied by an increase in the cardiac output of 30%-80%, suggesting that homoharringtonine caused reduction in peripheral vascular resistance leading to circulatory failures in its most severe form. There was no clear evidence of direct cardiac damage.

Myelosuppression

Myelosuppression was the second most significant toxic effect of homoharringtonine (table 4). The patients with solid tumors who had regular blood cell count determinations at the dose levels of 0.2 and 6 mg/m^2 were included in this analysis. No significant changes in blood cell counts were observed with dose levels < 1.5 mg/m^2. A reduction of platelet count < 150,000/mm^3 was observed in 21 of 44 patients (48%) and a wbc count < 4000/mm^3 or a granulocyte count < 2000/mm^3 was observed in 15 of 44 (34%) patients. Two patients had granulocyte counts drop < 500/mm^3 and two developed fever during neutropenia, requiring antibiotics. There was a wide variation in the degree of hematologic toxicity, with some patients experiencing significant lowering of blood cell counts in the dose range of 3-3.5 mg/m^2, whereas other patients had no significant effect on the blood cell counts until they reached dose levels of 5-6 mg/m^2. Thrombocytopenia was generally more frequent at each dose level and the platelet count depression was generally more severe than neutropenia. The lowest platelet count was < 50,000/mm^3 in five patients and < 20,000/mm^3 in two, both of whom required platelet transfusions. The median time to lowest granulocyte count was 14 days (range, 4-37) and the median time to lowest platelet count was 12 days (range, 7-39). The median time to recovery from myelosuppression was 21 days (range, 17-57). Twelve patients required > 28 days before they recovered from myelosuppression. The recovery period took > 3 weeks in most patients treated with 5-6 mg/m^2/day. The degree of thrombocytopenia appeared to be cumulative with repeated courses. Patients with extensive prior treatment had severe thrombocytopenia lasting 5-8 weeks before they recovered.

Nausea and vomiting were observed in a majority of the patients receiving homoharringtonine at the higher dose levels (table 5). Diarrhea, sometimes with associated abdominal cramps, occurred in some patients. Fever of 101°-103°F, associated with chills, occurred soon after completion of homoharringtonine infusions in a total of 11 patients. Mild stomatitis was observed in three patients. Several patients complained of marked weakness during the course of treatment.

Side Effects in Patients With Leukemia

Based on tolerance to a homoharringtonine dose of 5-6 mg/m^2/day in patients with solid tumors, five leukemia patients were treated in this dose range. Three of these patients experienced severe hypotension and shock requiring vasopressor support. Accordingly, subsequent patients were treated on a fixed dose of 4 mg/m^2/day and dose escalation was accomplished by a stepwise increase in the number of days of treatment as tolerated.

DISCUSSION

Homoharringtonine was selected for clinical evaluation in the United States, based on the reports of significant antileukemic activity in trials conducted in China. Complete remissions in acute leukemia were reported in 10%-20% of the patients, some of whom had not had any prior chemotherapy. Responses were also reported in Hodgkin's disease and nasopharyngeal carcinoma. Of the various dose schedules tested by the Chinese, a daily dose of 4-6 mg/day (approximately 2-4 mg/m^2) for several

TABLE 4.—Myelosuppressive effects of homoharringtonine

Dose (mg/m^2/day)	No. of patients*	Median lowest recorded counts × 10^3/mm^3 (range)		
		Wbcs	Neutrophils	Platelets
0.2-0.8	14	6.5 (2.9-19)	3.8 (1.9-17)	190 (52-386)
1.5	4	4.8 (4.1-7.8)	3.4 (2.2-5.8)	177 (95-295)
3.0-3.5	9	4.7 (1.7-8.2)	2.8 (0.8-5.6)	134 (44-182)
4.0-4.5	9	2.6 (1.4-6.0)	1.9 (0.6-4.6)	137 (18-325)
5.0-5.5	5	3.0 (0.8-6.4)	1.5 (0.2-2.0)	100 (9-130)
6.0	3	2.7 (3.2-2.6)	1.4 (0.8-1.9)	65 (37-115)

* Nos. of patients in this table differ from those in tables 2 and 3 because of exclusion of patients with inadequate blood cell counts and with acute leukemia.

TABLE 5.—Other toxic effects of homoharringtonine

Dose level (mg/m^2)	No. of patients						
	Total	Nausea or vomiting	Diarrhea	Fever	Dizziness	Alopecia	Stomatitis
0.2–0.8	14	2	0	—	—	—	—
1.5	6	2	2				
3.0–3.5	14	6	5	1	3	1	1
4.0–4.5	16	16	6	5	1	1	1
5.0–5.5	9	7	1	4	—	1	—
6.0	7	5	3	2	—	—	1
8.0	1	1	0	1	—	—	—

days was tolerable. However, the drug used in China consisted of mixtures of different alkaloids, making dose comparisons with more purified homoharringtonine difficult. This necessitated the undertaking of additional clinical phase I studies of the new preparation. Furthermore, cardiotoxicity was mentioned as a serious side effect of this drug, although no specific details besides sinus tachycardia and rhythm disturbances were available. Our phase I study using pure homoharringtonine revealed that a daily dose of 4–4.5 mg/m^2 for 5 consecutive days was the maximum tolerated safe dose. Patients with extensive prior treatment and those with poor performance status had significant side effects even at the 3-mg/m^2/day dosage. The dose-limiting toxic effect of homoharringtonine was hypotension, which was dose-related and became a serious problem when the daily dosage of homoharringtonine amounted to ≥ 5 mg/m^2. A moderately severe degree of myelosuppression was observed in a majority of patients, with thrombocytopenia being more common than leukopenia. Other side effects were quite similar to those previously reported and included mild nausea, vomiting, stomatitis, and diarrhea. We also observed a significant incidence of febrile reactions associated with the drug administration which have not been previously reported in the literature.

The reports of cardiac toxicity in the Chinese literature were of major concern during our phase I study. We observed that homoharringtonine caused peripheral vasodilatation resulting in hypotension. These vascular effects were slow in onset, generally occurring 4–6 hours later, and led to dizziness and tachycardia but without significant cardiac arrhythmias. Nearly all patients mounted a significant increase in cardiac output and required additional fluids for expansion of the intravascular volume. In its most severe form, the peripheral vasodilatation resulted in shock and its sequelae. Although three patients developed evidence of pulmonary edema during efforts at volume expansion, it is unclear whether the drug has any direct myocardial toxicity of the type seen with anthracycline drugs. The cardiovascular effects following a dose generally lasted for 24–48 hours. These effects were generally mild when the daily dosage was < 5 mg/m^2, which led us to limit the dose to no more than 4 mg/m^2/day. Any further increase in the dosage was accomplished by extending the duration of the treatment to an additional 1 or 2 days beyond the planned 5-day courses.

Although none of our patients achieved complete or partial response, several patients evidenced minor hints of antitumor activity. In order to further investigate the antitumor activity of this drug, we are planning phase II studies in the common solid tumors. In acute leukemia, we plan to initiate a phase I–II study to determine the optimum dose of the drug given over ≥ 10 days, until the drug has had its maximum antileukemic effect or limiting toxicity is reached. Since fractionation of the daily dose had a better antitumor effect in experimental animals, continuous infusion of homoharringtonine is also under investigation in several cancer centers (4,5). Early results of one such study indicate a lack of hypotension when the drug was given as a continuous infusion in the dose range of 3–4 mg/m^2 (4). Whether this schedule will be protective against hypotension at higher dose levels remains to be established. Since we and others have observed myelosuppression at 3–4 mg/m^2, the dose range which is free of hypotension, we have initiated phase II trials in solid tumors using these dose levels. Although the hypotensive effects of homoharringtonine can be very serious and potentially fatal, we believe that the drug can be used safely, provided the daily dose guidelines defined in this report are followed and adequate provisions made for monitoring of blood pressure and supplemental iv fluids are administered in patients who develop hypotension.

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Phase I Study of Weekly High-Dose Human Lymphoblastoid Interferon[1]

Joseph M. Connors* and Hulbert K. B. Silver[2,3]

Interferon has been shown to have antineoplastic activity but an optimal dose schedule has not been defined. In this phase I study 12 patients with advanced cancer were treated with weekly high-dose human lymphoblastoid interferon given by 3-hour iv infusion to assess toxicity. The median maximum tolerated dose was 55 × 10^6 units/m^2 and the dose-limiting toxicity was a complex of fever, fatigue, myalgias, anorexia, and weakness. Neither myelosuppression nor hepatotoxicity was encountered. One patient with metastatic melanoma achieved complete remission, which has been maintained for 2 years. Weekly high-dose iv infusion of interferon has antineoplastic activity, did not cause myelosuppression or hepatotoxicity, and was well-tolerated up to doses of 40–50 × 10^6 units/m^2. Future trials should start at 30 × 10^6 units/m^2 and should escalate by 10 × 10^6 units/m^2/week to patient tolerance. [Cancer Treat Rep 68:1093–1096, 1984].

The interferons are a family of glycoproteins with known antiviral and antineoplastic properties. In human studies various interferon preparations have been shown to have activity against multiple myeloma (1), nodular lymphoma (2), breast carcinoma (3), melanoma (4), renal cell carcinoma (5), ovarian carcinoma (6), and nasopharyngeal carcinoma (7). An optimal dose schedule has not been defined. Most investigators have tried various schemes emphasizing frequent administration. Typical regimens involve daily im injections, daily iv infusions, or continuous iv infusions for several days. Since the mechanism of antineoplastic activity of interferon is not known and therefore an optimal schedule cannot be selected on theoretical grounds, we decided to investigate a less frequent dosing schedule. Such a less frequent administration, if equivalently effective, would be much more attractive for routine clinical use. In addition, the dose-limiting toxicity may not be the same as with frequent administration and may permit intermeshing with standard chemotherapy.

MATERIALS AND METHODS

Twelve patients with locally advanced or metastatic cancer were entered in this study. Patients were eligible if they had an Eastern Cooperative Oncology Group performance status of 0 or 1; no symptomatic cardiovascular disease; normal wbc, granulocyte, and platelet counts; and normal renal and hepatic function tests. Preference was given to patients who had not received prior chemotherapy; however, several patients (table 1) had received one conventional course of chemotherapy. All antineoplastic treatment was stopped at least 4 weeks before study entry and only limited-field palliative irradiation to nonindex lesions was allowed during the study. All patients gave informed consent before study entry. The patient with nodular lymphoma had painful massive lymphadenopathy and had refused conventional chemotherapy.

Human lymphoblastoid interferon (Wellferon) was supplied by the Burroughs Wellcome Company through Pacific Isotopes and Pharmaceuticals of British Columbia. The interferon preparation consists of a highly purified mixture of α-interferons (80%–90%) extracted from the supernatant of a suspension culture of Sendai virus-stimulated lymphoblastoid cells derived from a Burkitt lymphoma cell line (Namalwa) (8). The interferon was given in 200 ml of normal saline infused iv over 3 hours. All patients were hospitalized overnight and vital signs monitored every 30 minutes for 4 hours then every 2–4 hours. Wbc count with differential, platelet count, hemoglobin, serum alkaline phosphatase, bilirubin, creatinine,

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TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Diagnosis	Previous treatment	
			Chemotherapy	IFRT*
1	80	Melanoma	No	No
2	33	Melanoma	No	No
3	63	Hypernephroma	Vinblastine	Yes
4	51	Nodular lymphoma	No	Yes
5	74	Lung—large cell cancer	No	Yes
6	59	Mesothelioma	No	No
7	66	Lung adenocarcinoma	No	Yes
8	72	Hypernephroma	No	No
9	71	Adenocarcinoma, unknown primary	No	No
10	69	Hypernephroma	Vinblastine	No
11	54	Lung—squamous cell cancer	No	Yes
12	26	Ewing's sarcoma	Vinblastine, doxorubicin, cisplatin, 5-FU	Yes

* IFRT = previous involved-field radiation therapy to primary tumor.

and SGOT were determined before and 24 hours after each dose of interferon.

Interferon was administered once each week and the dose was escalated by 10×10^6 units/m^2 with each treatment until the maximum tolerated dose (MTD) was reached or tumor progression precluded further assessment. For purposes of this study, MTD was defined as the highest dose received by the patient which did not result in unacceptable toxicity. In practice, MTD was dictated by the fever and myalgia syndrome that accompanies interferon. Other side effects are noted below but all were minor. Thus, the MTD for each patient reflected the maximum amount of interferon that could be tolerated without unacceptably unpleasant fever, chills, malaise, fatigue, and myalgias. Patients 1-3 were started at 10×10^6 units/m^2, Patients 4-6 were started at 30×10^6 units/m^2, Patients 7-9 were started at 40×10^6 units/m^2, and Patients 10-12 were started at 50×10^6 units/m^2. All patients received acetaminophen at a dose of 650 mg orally every 4 hours throughout the day of the interferon infusion. Nausea was treated with metoclopramide at a dose of 10 mg iv up to hourly whenever present. When the MTD was exceeded, dose reductions were made in decrements of 5 or 10×10^6 units/m^2 until the MTD was determined. Patients with stable or responding disease continued in the study, receiving the MTD at weekly intervals, until tumor progression.

RESULTS

Patient characteristics are shown in table 1. There were 11 men and one woman; ages ranged from 26 to 80 years, with a median of 66 years. The number of doses of interferon per patient ranged from two to nine. Only one patient received fewer than four doses; he was withdrawn because of progressive disease after receiving only two doses of interferon. The dose-limiting toxicity for the remaining 11 patients was a complex of chills, fever, myalgias, weakness, anorexia, and fatigue. Every patient developed fever, with peak temperatures ranging from 38.3°C to 40.3°C. Although the febrile reaction resolved in hours, the other dose-limiting symptoms often persisted 2-4 days after treatment. Table 2 shows the number of interferon doses each patient received and the MTD. The mean MTD ± 1 SD was $53 \pm 16 \times 10^6$ units/m^2 and the median MTD was 55×10^6 units/m^2. In general, there was no relationship between age and MTD with patients up to 72 years of age tolerating the same dose as those younger; however, the 74- and 80-year-old patients

TABLE 2.—No. of interferon doses and MTD

Patient No.	No. of interferon doses	MTD*
1	8	30
2	9	80
3	8	40
4	2	—
5	4	30
6	5	60
7	4	55
8	6	55
9	4	55
10	4	80
11	5	50
12	4	55

* $\times 10^6$ units/m^2 of body surface area.

could only tolerate 30 × 10^6 units/m^2. Since only patients with an excellent performance status were included, its impact on MTD could not be assessed. Too few patients had received any previous chemotherapy to assess its effect on MTD. Three of the four patients who tolerated less than the median MTD had received irradiation, whereas none of the three who tolerated more than the median MTD had been so treated.

Other, non-dose-limiting, mild toxic effects were encountered: six patients developed brief emesis; two, headache; one, aphthous ulcer; and one, transient vertigo. Every patient developed a transient drop in blood pressure, both systolic and diastolic, of between 10 and 30 mm Hg during interferon infusion. The hypotension resolved shortly after the infusion and did not cause symptoms or interfere with ambulation after infusion. Although not required to do so, most patients remained supine during the infusion and thus were not monitored for orthostatic symptoms. During the course of treatment patients lost an average of 2.9 kg (range, 0–7.0). No abnormality in renal function tests or liver function tests occurred in any patient. Cbcs were performed before and 24 hours after each dose of interferon. No abnormalities of wbc count, hemoglobin, or platelet count were detected. Specifically, the lowest wbc count recorded ranged from 3.1 to 7.4 × 10^9/L. No patient had an absolute granulocyte count < 1.6 × 10^9/L.

Tumor Response

Although this was a phase I study, it was possible to assess the antitumor effect of the interferon in all patients. One patient, an 80-year-old man with metastatic melanoma, achieved complete remission. Multiple regional subcutaneous and lymph node metastases, measuring up to 2 cm in diameter, resolved during 5 weeks of treatment. After eight doses his treatment schedule was changed to 30 × 10^6 units/m^2 of interferon iv over 3 hours every 2 weeks. He has maintained a complete remission for 2 years and has received no interferon or other treatment for the past year. He continued to have chills, fever, and myalgias with each interferon dose, but no other toxic effects were noted. Three patients had stable disease lasting 4–8 weeks. Eight patients had measurable or symptomatic progression of disease during their 2–5 weeks in the study.

DISCUSSION

Weekly high-dose iv infusions of human lymphoblastoid interferon may have certain advantages over the more typical frequent dosing schemes being investigated in most current trials. Since an optimal schedule for interferon has not been defined in terms of either efficacy or toxicity, there is a clear need for exploration of schemes which are more practical for outpatient use over extended periods of time. Interferon given as a weekly 3-hour infusion at a dose of 40–50 × 10^6 units/m^2 was acceptably tolerated by most patients, had antitumor activity, and did not cause bone marrow, hepatic, or renal toxicity in this study. The dose-limiting toxicity was a complex of symptoms including fever, chills, myalgias, fatigue, anorexia, and weakness. Moderate hypotension occurred during infusion, but promptly resolved and was asymptomatic in the supine patient. The dose for a given patient can be titrated to permit acceptable self-care and functioning at home.

The reported dose-limiting toxicity of frequently administered interferon (four to seven doses per week) consists of myelosuppression or hepatic enzyme abnormalities in addition to the usually noted complex of fever, myalgias, and fatigue (3). In a study of less frequent intermittent im recombinant leukocyte A interferon, Horning et al (9) found that the fever and fatigue complex was dose-limiting and that myelosuppression and liver enzyme abnormalities were minor. With the weekly iv infusion schedule reported in the present study, major toxicity was confined to the fever and fatigue complex. No myelosuppression or hepatic dysfunction was detected. This dissociation of toxic effects may be usefully exploited in future studies since myelosuppression is a common dose-limiting toxic effect for many standard chemotherapeutic agents. Interferon in weekly iv infusions might be added to such myelosuppressive agents or used when myelosuppression or hepatic dysfunction has been caused by the underlying malignancy. Careful monitoring will be necessary in any such trials since such patients may be unlike our patients who had normal hematopoietic function on entry and received only interferon.

Weekly iv interferon induced a complete remission in one patient with metastatic melanoma. This patient then received treatment once every 2 weeks for 1 year and has been off treatment an additional year still in complete remission. The only toxic effect was 1 day of fever and fatigue with each treatment. This demonstrates that interferon administered at weekly intervals can have biologic antineoplastic activity. However, it will be necessary to test interferon in prospective trials comparing different dose schedules before an optimal antineoplastic regimen can be defined.

For future testing of weekly high-dose iv interferon, we recommend a starting dose of 30 × 10^6 units/m^2 given over 3 hours to a supine patient. Doses should be escalated to individual tolerance at a rate of 10–20 × 10^6 units/m^2/week then maintained at that dose. Patients with previous radiation therapy may require somewhat lower doses. Phase II testing should be conducted in patients with metastatic melanoma and those other tumor types known to be responsive to interferon on other dose schedules. In our experience weekly high-dose iv interferon is a practical, potentially effective regimen without hematologic or hepatic toxicity when given to patients

with minimal or no prior chemotherapy. It should be tested further for specific tumor activity.

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Phase I Clinical Evaluation of Cytarabine and Cisplatin in Patients With Advanced Cancer[1]

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Studies in cell culture systems and tumor-bearing animals have demonstrated synergistic cytotoxicity of cytarabine (ara-C) and cisplatin. We have conducted a phase I trial to assess the toxic effects and tolerable doses of these drugs in patients with advanced cancer. Forty-five such patients were treated with varying dosages of ara-C infused continuously during Days 1–3 of a 28-day cycle. Cisplatin at a dose of 100 mg/m² was administered on Day 2 of the cycle. Using this schedule, the maximally tolerated dose of ara-C in previously untreated patients was 60 mg/m²/day (180 mg/m²). Hematologic toxicity was dose-limiting with median wbc and granulocyte count nadirs of 1800 and 168/mm³, respectively. Reduction of the cisplatin dose while maintaining the ara-C dose at 60 mg/m²/day resulted in less myelosuppression, suggesting that these drugs may have synergistic effects on the bone marrow. Objective responses were seen in six of 41 evaluable patients, including five of 12 patients with non-small cell lung cancer. The severe bone marrow toxicity observed at relatively low drug doses and the 42% response rate in patients with non-small cell lung cancer suggest that the combination of ara-C and cisplatin has substantial clinical activity. Phase II trials are warranted in non-small cell lung cancer and other tumors. [Cancer Treat Rep 68:1097–1102, 1984]

Since its introduction into clinical trials in 1972, cisplatin has become a valuable drug in the treatment of a broad spectrum of solid tumors. In combination with vinblastine and bleomycin, cisplatin is curative in the majority of patients with non-seminomatous germ cell tumors of the testis (1) and has established clinical activity in the treatment of ovarian, head and neck, and non-small cell lung cancer, and in other tumors as well (2,3).

These impressive clinical results may be explained in part by studies in experimental systems which demonstrate that cisplatin is synergistic with a number of other commonly used antineoplastic agents (4–8). Among these, cytarabine (ara-C) has consistently exhibited synergistic cytotoxicity with cisplatin in both in vitro and in vivo systems. The most dramatic effects have been observed by Bergerat et al (9) using cultured human colon carcinoma cells (LoVo cells). Exposure of these cells to ara-C in concentrations of 100–1000 μg/ml plus cisplatin in concentrations of 0.1–10 μg/ml produced a dose-related 1–2-log increase in cell kill beyond that predicted to result from a simple additive effect of the drugs. This synergy was particularly impressive since ara-C alone, even in high doses, failed to produce any lethal effects in LoVo cells.

Although ara-C and cisplatin are each commonly used in clinical practice and have well-defined indications and toxic effects, the drugs have rarely been used together and the toxic effects to be expected from their combination have not been defined. We have therefore conducted a phase I clinical trial of ara-C/cisplatin with the objectives of defining the toxic effects of this combination and searching for evidence of drug synergism in man.

PATIENTS AND METHODS

Patient Selection

Patient characteristics are shown in table 1. Forty-five patients, 39 men and six women ranging from 35 to 74 years of age, were entered in the study. All patients had a

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total (evaluable)	45 (38)
Men	39
Women	6
Median age in yrs (range): 60 (35–74)	
Performance status*	
0	2
1	18
2	23
3	2
Prior therapy	
Chemotherapy	12
Chemotherapy + radiation therapy	7
Radiation therapy	7
None	19
Tumor types	
Non-small cell lung cancer	13
Carcinoma of head and neck	6
Adenocarcinoma of the colon	3
Adenocarcinoma of unknown primary	6
Small cell lung cancer	5
Renal cell carcinoma	3
Undifferentiated carcinoma of unknown primary	4
Non-Hodgkin's lymphoma	2
Adenocarcinoma of the breast	1
Osteogenic sarcoma	1
Sarcoma	1

* Cancer and Leukemia Group B criteria.

pathologically confirmed diagnosis of cancer and, in each case, the disease had proven refractory to standard therapy or was one for which no therapy of proven benefit was available. Twenty-six patients had been previously treated with radiation therapy, chemotherapy, or combined modalities, and 19 patients had received no prior therapy. Each patient had an initial complete medical history and physical examination, chest x-ray, electrocardiogram, urinalysis, cbc and determination of serum chemistries, and 24-hour urine creatinine clearance. Cbcs and platelet counts were then determined weekly during therapy while all other parameters were repeated on Day 1 of each cycle. Prior to beginning ara-C/cisplatin therapy, all patients had normal renal and liver function (serum creatinine < 1.8 mg/100 ml, creatinine clearance > 60 ml/minute, and total bilirubin < 1.5 mg/100 ml), a wbc count $> 4000/mm^3$, and a platelet count $> 100,000/mm^3$. The treatment protocol was approved by the Institutional Review Boards of the University of Missouri Health Sciences Center and the Harry S Truman Veterans Administration Hospital and all patients provided written informed consent for participation in the study.

Treatment Plan

The treatment protocol was designed based on the drug concentrations demonstrated to produce synergy in vitro and the known clinical pharmacology of cisplatin and ara-C. Following a rapid iv injection of cisplatin at 100 mg/m^2, peak plasma levels of 1–5 $\mu g/ml$ are obtained (10,11). These levels are well within the range of cisplatin concentrations found to produce synergistic cytotoxicity in vitro. Pharmacologically achievable levels of ara-C, however, are substantially below those found effective during a 1-hour exposure in the LoVo cell system. Ho and Frei (12), for example, observed plasma levels of only 0.05–0.1 $\mu g/ml$ in patients receiving ara-C infusions of 112 mg/m^2/day. Peak plasma levels as high as 100 $\mu g/ml$ can be obtained, but only after iv injection of several grams of the drug (13). Since the toxic effects of ara-C are related to both the dose employed and the duration of drug exposure, however, we postulated that clinically evident interactions with cisplatin might occur in vivo if exposure to lower ara-C doses were prolonged.

Patients were therefore treated with a continuous iv infusion of ara-C for 3 days and cisplatin, at a dose of 100 mg/m^2, administered iv over 1–2 hours on Day 2 of the cycle. Prior to cisplatin administration, patients were hydrated with 2 L of iv fluids and diuresis was established by rapid iv injection of 12.5 g of mannitol. Following cisplatin infusion, patients received 500 ml of 0.45% NaCl containing 12.5 g of mannitol and 20 mEq of KCl, after which urine output was replaced with fluids for an additional 24 hours. The initial dose of ara-C was 20 mg/m^2/day. Dosage escalation was carried out after at least three patients had been treated at each ara-C dose chosen for evaluation. There was no dose escalation in individual patients. Cycles of chemotherapy were repeated every 28 days. Patients were treated for at least two cycles of therapy unless rapid disease progression or unacceptable toxic effects occurred.

Criteria of Response

Although the primary objective of this study was to determine the nature and magnitude of ara-C/cisplatin toxicity, patients with clearly measurable lesions were evaluated for response to treatment. Standard response criteria were employed as follows: *complete response*—disappearance of all measurable disease, signs, symptoms, and biochemical changes related to the tumor for > 4 weeks and no appearance of new lesions; *partial response*—a reduction of $> 50\%$ in the sum of the products of the perpendicular diameters of all measurable lesions lasting > 4 weeks and no appearance of new lesions or enlargement of existing lesions; *minor response*—a reduction of $< 50\%$ but $> 25\%$ in the sum of products of the perpendicular diameters of all measurable lesions lasting > 4 weeks and no appearance of new lesions or enlargement of existing lesions; *stable disease*—$< 25\%$ change in the sum of the products of the perpendicular diameters of measurable lesions and no appearance of new lesions; and *progressive disease*—an increase in the product of the

perpendicular diameters of any measurable lesions by > 25% or the appearance of new lesions.

RESULTS

Toxic Effects

Forty-five patients were treated with ara-C/cisplatin chemotherapy during this study. Three patients died due to disease progression prior to completion of a full cycle of therapy and are therefore not evaluable. Three patients failed to obtain interim blood cell counts and another patient failed to meet minimum pretreatment criteria for adequate bone marrow function; all four were excluded from analysis. The remaining 38 patients received 80 cycles of ara-C/cisplatin, of which six were not evaluable for toxicity due to failure to obtain interim blood cell counts. Thus, 38 patients and 74 cycles of chemotherapy are evaluable for toxicity.

The major clinical toxic effect observed in this trial was bone marrow suppression. As shown in table 2, ara-C, in combination with cisplatin, produced myelosuppression at doses as low as 20 mg/m^2/day in some patients. Escalation of the ara-C dose was associated with more profound nadir counts, such that at 60 mg/m^2/day, all patients developed severe neutropenia with the median granulocyte count nadir being only 168/mm^3. Of note is that no patient treated at this dose level had previously received chemotherapy. While toxicity to granulocytes was severe and dose-related, platelet suppression was generally moderate and not clearly related to the administered ara-C dose.

The blood cell count nadirs occurred in a predictable biphasic fashion. Thrombocytopenia developed initially and was most severe by Day 10 of the treatment cycle. Neutropenia was more delayed in onset, with the nadir occurring in all patients between Days 17 and 20. Platelet and granulocyte counts generally returned to normal by Days 17 and 24, respectively.

These data strongly suggested that the combination of ara-C and cisplatin produces more bone marrow toxicity than would be expected from either drug alone or from an additive effect of the drugs. However, to exclude the possibility that the observed myelosuppression was due solely to the 72-hour ara-C infusion, the ara-C dose was fixed at 60 mg/m^2/day for 3 days and the cisplatin dose was progressively reduced. The hematologic toxicity observed in patients so treated is shown in table 3. Overall, 14 patients received 29 cycles of chemotherapy at an ara-C dose of 60 mg/m^2/day and a cisplatin dose of $\leq$ 70 mg/m^2. These patients did not differ significantly in age or performance status from those treated with cisplatin at 100 mg/m^2 and ten of the 14 patients had not previously received chemotherapy. The median wbc and granulocyte count nadirs for the entire group were 2900 and 1280/mm^3, respectively. These values are clearly substantially higher than those observed in the comparable group of patients treated at the same ara-C dose but a higher cisplatin dose and indicate that the profound neutropenia observed in the latter patients is not likely due to the effects of ara-C alone.

As shown in table 3, considerable variability in blood cell count nadirs did occur among patients treated at the lower cisplatin doses, although at each dose level evaluated, patients experienced less severe marrow toxicity than was observed in patients treated with cisplatin at 100 mg/m^2. There were no major differences in patient characteristics or extent of prior therapy which could be identified to explain the more severe toxicity experienced by the group treated with cisplatin at 50 mg/m^2, although it is conceivable that subtle differences in renal function, ara-C metabolism, or timing of drug administration could account for the more severe toxicity observed in these patients compared to those treated with cisplatin at 70 mg/m^2.

Other toxic effects observed in this trial were predictable and included gastrointestinal upset and altered renal function. Nausea and vomiting occurred in all patients and was particularly severe on the day of cisplatin administration. This unpleasant side effect was successfully managed in most patients with aggressive antiemetic therapy. Nephrotoxicity was not prominent in this study. Median pretreatment creatinine clearance for patients receiving cisplatin at 100 mg/m^2 was 82 ml/minute (range, 60–125). This fell to a median of 77 ml/minute (range, 38–140) during therapy. In six patients, creatinine clearance remained at or above pretreatment values, while the average decrease in creatinine clearance in

TABLE 2.—Hematologic toxicity at cisplatin dose of 100 mg/m^2

Ara-C dose (mg/m^2/day)	No. of patients	No. of cycles	Median nadir count (range)		
			Wbc*	Polymorphonuclear neutrophil	Platelet*
20	3	6	2.9 (1.3–6.8)	1073 (240–5016)	88 (21–265)
30	4	8	3.2 (1.2–6.2)	792 (165–3471)	303 (171–520)
45	10	20	2.1 (1.1–7.0)	392 (38–5180)	82 (20–510)
60	8	11	1.8 (0.8–4.4)	168 (18–1980)	90 (43–409)

* × 1000/mm^3.

TABLE 3.—Hematologic toxicity at ara-C dose of 60 mg/m²/day

Cisplatin dose (mg/m²)	No. of patients	No. of cycles	Median nadir count (range)		
			Wbc*	Polymorphonuclear neutrophil	Platelet*
100	8	11	1.8 (0.8–4.4)	168 (18–1980)	90 (43–409)
70	7	14	3.9 (1.6–10.5)	1504 (117–8295)	215 (9–366)
50	5	10	2.5 (1.1–5.4)	544 (11–4320)	145 (84–322)
25	2	5	3.0 (2.7–5.2)	1428 (972–4029)	185 (121–288)

* × 1000/mm³.

the remaining patients was 31% of the pretreatment value.

Another noteworthy toxic effect which appeared during this study was severe asthenia, occurring most commonly in patients who received ara-C at 60 mg/m²/day with cisplatin at 100 mg/m². Seven of eight patients treated at this dose level complained of profound malaise, weakness, and anorexia which persisted following the chemotherapy infusion, frequently long after complete hematologic recovery had occurred. These nonspecific complaints were not associated with electrolyte, hepatic, or metabolic disturbances and generally resolved over a period of 3–5 weeks. However, one patient refused to continue with chemotherapy due to the severity of these symptoms and, in another, the interval between chemotherapy treatments was extended to allow for recovery.

There was one treatment-related death during this study. A patient with adenocarcinoma of unknown primary site metastatic to lymph nodes and lung received ara-C at 60 mg/m²/day and cisplatin at 100 mg/m². She returned to the hospital on Day 15 of her treatment cycle with fever and a total wbc count of 800/mm³ with 24% granulocytes. Though no source of sepsis was clearly identified, it is likely that this drug-induced bone marrow toxicity contributed to her stormy hospital course and death.

Response Data

Overall, six of 41 patients (15%) with measurable disease obtained complete or partial remissions with ara-C/cisplatin chemotherapy. These data are summarized in table 4. Responses were seen at several ara-C + cisplatin dose levels, including 45 mg/m² + 100 mg/m², 60 mg/m² + 100 mg/m², 60 mg/m² + 70 mg/m², and 60 mg/m² + 50 mg/m². Of particular interest is the response rate of 42% in patients with non-small cell lung cancer. Clinically important disease stabilization also occurred in one patient with extensive small cell lung cancer who developed progressive disease while receiving conventional multidrug chemotherapy. This individual had extensive bone and pulmonary disease yet remained clinically stable and able to carry on usual daily activities for 6 months while receiving chemotherapy before developing intracerebral metastases and progressive liver disease.

A complete response was observed in one patient with locally recurrent squamous cell carcinoma of the head/neck. A biopsy-proven neck recurrence completely disappeared after two cycles of therapy and re-biopsy of the involved area revealed no evidence of tumor. The patient received a third cycle of therapy and subsequently remained free of disease off all therapy for 4.5 months before relapse occurred.

TABLE 4.—Response data

Diagnosis	No. of patients*					
	Total (evaluable)†	CR	PR	MR	SD	PD
Non-small cell lung cancer	13 (12)	0	5	1	0	6
Head/neck cancer	6 (5)	1	0	0	0	4
Small cell lung cancer	5 (5)	0	0	1	1	3
Lymphoma	2 (1)	0	0	1	0	0
Adenocarcinoma, unknown primary	6 (5)	0	0	0	1	4
All others	13 (13)	0	0	0	0	13
Total	45 (41)	1	5	3	2	30

* CR = complete response; PR = partial response; MR = minor response; SD = stable disease; and PD = progressive disease.
† 3 patients not evaluable for toxicity were evaluable for response.

DISCUSSION

The toxic effects of ara-C are related to both the administered dose and the duration of drug exposure (14-16). Early clinical studies of this drug failed to demonstrate bone marrow toxicity following single iv bolus doses as high as 4 g/m^2 (16). With continuous iv infusion for 48-96 hours, however, neutropenia became apparent at doses of 450 mg/m^2 and was dose-limiting at 1000 mg/m^2 (16).

The primary clinical toxic effects of cisplatin are related to its effects on renal function and the gastrointestinal tract (3). Bone marrow suppression is usually mild and transient in patients receiving this drug as a single agent. Generally, only 20%-40% of patients treated with cisplatin in doses as high as 100 mg/m^2 experience leukopenia, which is usually in the range of 2000-3000/mm^3 (3,17).

The bone marrow toxicity observed in the present study, then, is substantially more than would be expected to result from an additive effect of these drugs at the doses used and suggests that these agents may indeed act synergistically in man. In previously untreated patients, the maximally tolerated dose of ara-C in combination with cisplatin is only 180 mg/m^2 infused continuously for 72 hours and, in patients who had previously received chemotherapy, it is only 135 mg/m^2. The granulocyte series is primarily affected and neutrophil counts fall in a dramatic and dose-related fashion as the ara-C dose is increased. Reduction of the cisplatin dose ameliorates the bone marrow toxicity such that at an ara-C dose of 60 mg/m^2/day, the median granulocyte count nadir observed in patients treated with $<$ 100 mg/m^2 of cisplatin is more than seven times higher than that seen at a cisplatin dose of 100 mg/m^2. Indeed, only minimal bone marrow toxicity was observed in two patients when the cisplatin dose was reduced to 25 mg/m^2. Thus, dose levels of ara-C/cisplatin can be identified at which only minimal marrow toxicity occurs, demonstrating that neither drug alone, at the doses employed, is capable of producing the profound myelosuppression observed in this study.

The clinical responses observed during this trial provide some additional evidence for the activity of this regimen. Early clinical trials of ara-C failed to demonstrate any antitumor activity in patients with non-small cell lung cancer (15,16,18). Cisplatin appears to be only modestly active, with response rates of 15%-20% reported in patients with these tumors (3,19). Our observation of five partial remissions in 12 patients with non-small cell lung cancer is therefore encouraging and suggests that ara-C and cisplatin may have substantial antitumor activity in this disease. Further studies are necessary to more accurately define the response rate of this combination in patients with lung cancer.

The mechanism of ara-C/cisplatin synergy is presently unknown, although several hypotheses have been advanced to explain it. Bergerat et al (9) have speculated that ara-C enhances cisplatin-induced DNA strand cross-linking or that ara-C inhibits cellular repair of cisplatin-induced DNA damage. While some experimental data exist to support the former theory, further in vitro studies are clearly necessary to more completely define the nature of this drug interaction at the molecular level.

This phase I study strongly suggests that ara-C and cisplatin produce synergistic cytotoxicity in patients with advanced cancer. Phase II studies in patients with lung cancer, head and neck cancer, and lymphoma are warranted to further define the spectrum of clinical activity of this drug combination. Recommended starting doses for phase II studies are 45 mg/m^2/day of ara-C and 100 mg/m^2 of cisplatin on Day 2.

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Phase I Clinical and Pharmacologic Trial of Carboplatin Daily for 5 Days[1,2]

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Twenty-two patients with refractory tumors received 64 courses of iv bolus carboplatin every day × 5, every 4–5 weeks. All patients are evaluable for toxicity and 18 are evaluable for response. For solid tumor phase II studies, a dose of 77 mg/m²/day × 5 is recommended for patients who have received prior chemotherapy. Patients with no prior chemotherapy experience should receive 99 mg/m²/day × 5. The major dose-limiting side effect is dose-related thrombocytopenia. Courses of carboplatin on this schedule should be repeated every 5 weeks to avoid cumulative wbc count toxicity, since leukopenia frequently did not occur until Day 28. Other toxic effects observed were nausea and vomiting and lower-extremity myalgias and arthralgias. There was no evidence of hearing loss, mucosal damage, or changes in liver or renal function tests of any patient while in this study. Therapeutic responses were seen in four patients: one partial response in renal cancer of 9+ months' duration; one partial response in head and neck cancer of 7+ months' duration; and objective responses in melanoma and colorectal carcinoma of 6 months' duration. [Cancer Treat Rep 68:1103–1114, 1984]

Cisplatin (*cis*-DDP) has demonstrated significant antitumor activity against a number of animal and human malignancies (1-4). However, its utility is limited by renal toxicity and severe nausea and vomiting. It is therefore important that analogs of *cis*-DDP be developed and studied to obtain other active but less toxic compounds. Carboplatin (fig 1) interacts in vitro with PM-2 DNA to produce a DNA shortening effect and suprahelical conformation changes (1-7). Carboplatin is active against several National Cancer Institute (NCI) tumor panel malignancies including intraperitoneal B16 melanocarcinoma, subcutaneous CD8F1 mouse mammary carcinoma, murine colon 26 adenocarcinoma, and human breast carcinoma xenografts implanted under the renal capsule of athymic mice. Carboplatin was more active by the ip route when given every 6 hours on a Day-1, -5, and -9 schedule when tested against murine P388 murine leukemia (8). Animal toxicology studies revealed that carboplatin was considerably less emetigenic and nephrotoxic than *cis*-DDP. However, the major dose-limiting toxic effect of carboplatin was myelosuppression. A clinical phase I study performed in England and employing a single-dose iv bolus schedule indicated that thrombocytopenia was the most common clinical side effect of carboplatin. In addition, carboplatin produced moderate nausea and vomiting and no renal toxicity or ototoxicity (9). Antitumor activity was seen against ovarian carcinoma.

The current study was undertaken to determine the maximum tolerated dosage (MTD) of carboplatin when given as a single iv bolus daily for 5 days. Courses were repeated every 4-5 weeks and pharmacokinetic studies were performed on all patients entered at doses of 11-77 mg/m².

MATERIALS AND METHODS

To be eligible for this protocol, patients had to fulfill the following criteria. All patients had histologic proof of a malignant disease which had failed conventional chemotherapy or for which no such conventional therapy existed. Patients had recovered from all toxic effects from prior treatments and were at least 4 weeks beyond any prior chemotherapy or radiotherapy. All patients had a minimum life expectancy of 12 weeks and a performance status of at least 60% on the Karnofsky scale.

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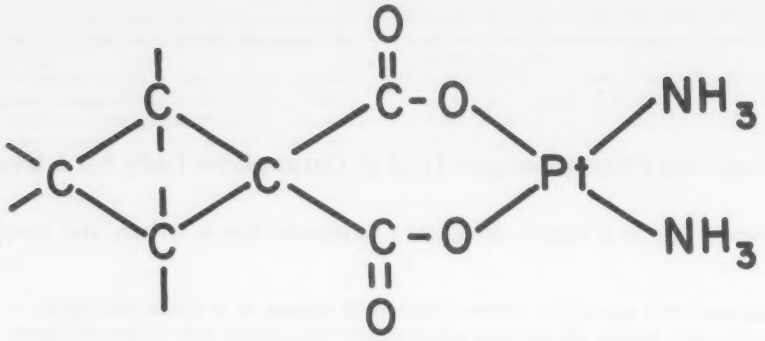

FIGURE 1.—Structure of carboplatin.

All patients had evidence of adequate bone marrow function (wbc counts > 3500 cells/μl and platelet counts > 100,000/μl), adequate liver function (bilirubin < 2.0 mg/dl), and adequate renal function (serum creatinine < 2.0 mg/dl and a 24-hour creatinine clearance of > 60 ml/minute). Objective measurable disease was desirable but not required. All patients entering the study were informed of the investigational nature of this treatment, the results that might be expected, and the toxic effects which might reasonably be anticipated.

Carboplatin is an investigational agent supplied by the National Cancer Institute (Bethesda, MD) in 20-ml amber vials containing a sterile freeze-dried powder consisting of 150 mg each of carboplatin and mannitol. The powder was dissolved in sterile water for injection to yield a solution containing 15 mg/ml of carboplatin and 15 mg/ml of mannitol at a pH of 5.5. In this study, carboplatin was given as an iv bolus injection daily for 5 days. Courses were initially repeated every 4 weeks. Later in the study, this period was extended to 5 weeks to allow for recovery from hematologic toxicity. The starting dosage of carboplatin was based on animal toxicology data supplied by the NCI, and was one-tenth of the LD10 in dogs. A cohort of three patients was studied at the starting dosage. An interval of 1 week passed between the entry of each of the patients at this treatment level. Subsequent patients were entered at higher dosages if no dose-limiting toxicity had occurred at the previous dosage. New dosages were chosen with a modified Fibonacci search scheme. A minimum of two patients previously untreated with carboplatin were entered at each dosage level. Dosages were escalated within the same patient after the patient had received two courses of carboplatin with mild reversible toxicity. Six or more patients were studied at dosages which produced acceptable, reversible toxicity. Patients did not receive any antiemetics during their first course of carboplatin so that this side effect might be adequately evaluated.

On each treatment day and weekly thereafter, serum tests including sodium, potassium, chloride, magnesium, phosphorus, BUN, and creatinine were measured. Twenty-four-hour urine creatinine clearances were performed prior to carboplatin therapy and every 2 weeks thereafter in all patients. Serum liver function tests were done prior to therapy and then weekly while in the study. Electrocardiograms and chest roentgenograms were done prior to entry in the study and as indicated thereafter. Audiograms were measured prior to carboplatin therapy and then every 8–9 weeks. All of the above examinations were repeated when a patient was removed from the study.

Pharmacokinetic Studies

At specified times after drug administration, blood samples were collected in heparinized tubes, centrifuged at 1000 × *g* for 10 minutes, and the plasma was removed. An aliquot of plasma was frozen at -20°C until the time of assay. Protein-free ultrafiltrates were prepared by centrifuging 3 ml of the remaining plasma in Centriflo CF50A membranes (Amicon Co, Lexington, MA) at 1000 × *g* for 20 minutes at 4°C. Urine samples were collected at the time of voiding, measured, and stored at -20°C as pooled 4-hour collections.

Plasma, ultrafiltrates, and urine samples were analyzed for platinum by flameless atomic absorption spectrometry, employing the same instrumentation and methodology previously reported (10).

Plasma pharmacokinetics were analyzed with the programs of MLAB (an on-line modeling laboratory, Division of Computer Resources and Technology, National Institutes of Health, Bethesda, MD) (11). Curves were fit to the sum of two exponentials by a nonlinear technique with and without weighting by $1/SE^2$. Concentrations times time (C × T) for the curve of ultrafilterable platinum in plasma was estimated by calculating the area under curve (AUC) by means of the integral operator in MLAB. In addition, AUC, total-body clearance (CL_{TB}),

TABLE 1.—Diagnoses and therapeutic responses in patients treated with carboplatin

Tumor type	No. of patients	No. of evaluable patients*	No. of responses†	Duration (mos)
Melanoma	2	2	1 OR	6
Renal cell cancer	2	1	1 PR	9+
Colorectal cancer	6	6	1 OR	6
Pancreatic cancer	1	1	1 stable disease	3
Lung cancer				
Alveolar cancer	1	1	1 stable disease	6
Squamous cancer	1	1	1 stable disease	8
Adenocarcinoma	2	2	—	—
Head and neck cancer	2	1	1 PR	7+
Ovarian cancer	1	1	—	—
Cervical cancer (adenocarcinoma)	1	1	—	—
Vaginal (squamous) cancer	1	1	—	—
Plasmacytoma	1	0	—	—
Breast cancer	1	0	—	—
Totals	22	18		

* Measurable disease and at least 2 courses of carboplatin.
†PR = partial response; OR = response less than partial.

and steady-state volume of distribution (Vd_{SS}) were calculated by noncompartmental methods, available in MLAB.

RESULTS

Twenty-two patients were entered in this phase I clinical and pharmacologic study and received 64 evaluable courses (median, two; range, one to eight) (table 1). All patients were evaluable for toxicity and 18 were evaluable for response. Three patients received one course, nine received two courses, five received three courses, two received five courses, and one each received four, six, and eight courses. There were ten males and 12 females, with a median age of 55 years (range, 40–71) and a median performance status of 80% (range, 60%–100%). Prior to entry in this study, six patients had received chemotherapy alone, five had received chemotherapy plus radiation therapy, four had received radiation therapy only, three had received interferon plus chemotherapy plus irradiation, and one had received chemotherapy plus whole-body hyperthermia. Three patients had received no prior antineoplastic therapy. Nonhematologic toxicity (table 2) was confined to emesis, which was dose-related, and lower-extremity myalgias and arthralgias, noted at the highest dosage level. Nausea was first encountered at the 55-mg/m²/day level. This symptom gradually increased both in frequency and in severity until the 99-mg/m²/day level, where nine of 11 patients experienced some sort of gastrointestinal disturbance. One patient treated at 99 mg/m²/day was admitted to the hospital for dehydration secondary to emesis. The only other nonhematologic side effect, lower-extremity myalgias beginning approximately 12 hours after each dose of carboplatin and persisting for 8–12 hours, occurred in two of ten patients treated with 99 mg/m²/day. This musculoskeletal discomfort occurred only on treatment days and was reproducible with repeated courses of carboplatin. There was no evidence of hearing loss, mucosal damage, or changes in liver or renal function tests of any patient while in this study.

TABLE 2.—Nonhematologic toxic effects occurring in patients treated with carboplatin

Dose (mg/m²/day × 5)	No. of patients	Nausea and vomiting*	Other
11	3	0	0
22	2	0	0
55	7	2/1	0
77	12	5/1	0
		4/2	
		1/3	
99	11	2/0	2 patients with lower-extremity myalgias and arthralgias
		5/1	
		3/2	
		1/3	

* No. of patients/toxicity grade (Cancer and Leukemia Group B scale).

TABLE 3.—Hematologic toxic effects occurring in patients treated with carboplatin

Dose (mg/m^2/day × 5)	No. of patients		Wbc count		Platelet count	
	Evaluable	With previous carboplatin	Nadir (× $10^3/\mu l$)	Nadir Day* (range)	Nadir (× $10^3/\mu l$)	Nadir Day* (range)
11	3	0	All > 3.5	—	1 patient, 64 (disease-related)	31
22	2	0	All > 3.5	—	All > 100	—
55	7	4	4.5 (2.2-6.8)	18 (7-31)	124 (42-260)	24 (21-30)
77	12	5	3.5 (0.8-5.1)	30 (6-42)	89 (7-246)	23 (16-29)
99†	5	3	2.7 (2.1-2.4)	30 (12-41)	67 (40-111)	23 (22-24)
99‡	6	1	3.4 (2.6-7.0)	26 (16-34)	125 (48-172)	23 (16-31)

* Nadir days for those courses with wbc counts < 3.5 × $10^3/\mu l$ and platelet counts < 100,000/μl.
†Prior chemotherapy.
‡No prior chemotherapy.

Hematologic toxic effects secondary to carboplatin treatment are shown in table 3. The dose-limiting toxic effect was dose-related thrombocytopenia. For patients who had received any prior chemotherapy, the median platelet count nadir was 89,000/μl at 77 mg/m^2/day and 67,000/μl at 99 mg/m^2/day. However, for patients who had received no prior chemotherapy, a dose of 99 mg/m^2/day was easily tolerated and, at this level, three of six patients had platelet count nadirs > 100,000/μl. The median time to platelet count nadir was approximately 3 weeks but the median time to wbc count nadir at the higher dosage levels was 4 weeks. Platelet counts usually recovered by Day 28 and wbc counts usually recovered by Day 35. For this reason, all subsequent carboplatin treatments were administered every 35 days. One patient required hospitalization for presumed, but not documented, infection with fever and granulocytopenia related to carboplatin treatment while in this study. No patient experienced bleeding related to hematologic toxicity from carboplatin. Patients escalating to higher carboplatin dosage levels had no more myelosuppression than did new patients entered at these levels.

Therapeutic benefit was seen in seven of 18 patients evaluable for response (table 2). Stable disease lasting 3-8 months occurred in three patients. Objective responses which were less than partial responses and which lasted for 6 months were observed in one patient with malignant melanoma and in one patient with colon cancer. Partial responses were seen in patients with renal cell carcinoma and head and neck cancer.

Pharmacokinetics

Plasma concentrations of total platinum declined in a biexponential fashion with alpha and beta half-lives of 26-49 and 492-1188 minutes, respectively (fig 2; table 4). As expected, administration of higher doses of carboplatin produced proportionally higher peak plasma concentrations and AUCs for total plasma platinum (fig 2; table 4). There were, however, no consistent effects of dosage on plasma half-life or CL_{TB} of plasma total platinum, the latter of which approximated creatinine clearance (table 4). As might be expected from these values and the doses administered, there were minimal amounts of platinum in plasma 24 hours after administration of carboplatin. In 14 of 15 courses, there was no accumulation of platinum in plasma samples obtained before treatment on Days 2-5 of each course. In one patient with carcinoma of the breast and massive ascites, there were progressively higher concentrations of plasma total platinum measured before administration of carboplatin on Days 2-5. These concentrations, however, never exceeded 0.3 $\mu g/ml$.

Plasma concentrations of ultrafilterable platinum declined in biexponential fashion (fig 3; table 5), with alpha and beta half-lives of 7.6-21.4 and 102-236 minutes, respectively, ie, threefold to fivefold less than those for concentrations of total plasma platinum. Again, administration of higher doses of carboplatin produced proportionally higher peak plasma concentrations and AUCs (figs 3 and 4; table 2) for ultrafilterable platinum, but had no effect on its plasma half-life, CL_{TB}, or Vd_{SS} (table 5). As expected, the CL_{TB} of ultrafilterable platinum exceeded that for total plasma platinum, being approximately threefold to fourfold greater (tables 4 and 5).

Unlike that in the patients receiving *cis*-DDP, most platinum in the plasma of patients receiving carboplatin was ultrafilterable (fig 5). Ultrafilterable platinum accounted for ≥ 70% of the total plasma platinum for the first 2 hours after iv bolus injection of carboplatin, after which this percentage slowly declined to approximately 50%. This pattern was the same at all four dosages of carboplatin studied. Unfortunately, we did not have measurements of plasma ultrafilterable platinum at times between 6 and 24 hours after carboplatin injection and, as mentioned above, by 24 hours after injection, little if any platinum remained in the plasma.

The kidney was the major route of excretion of platinum in patients receiving carboplatin, with most excretion occurring within the first 8 hours after drug administration (fig 6). By 24 hours, 53.6% ± 5.6% of the platinum injected had been excreted in the urine. Similar pat-

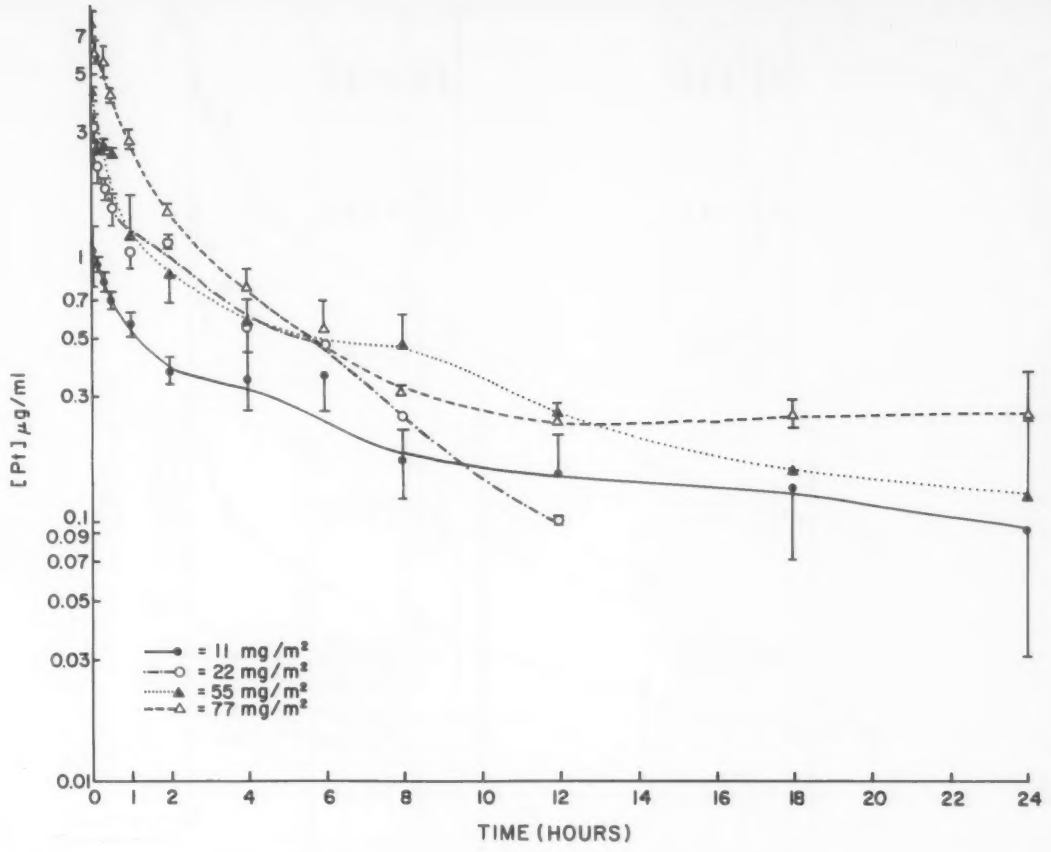

FIGURE 2.—Concentrations of platinum in plasma of patients treated with carboplatin. Carboplatin was injected as an iv bolus and plasma platinum was determined by flameless atomic absorption spectrometry. Points represent means ± SE of 4, 3, 3, and 5 courses administered to 3, 2, 3, and 5 patients at doses of 11, 22, 55, and 77 mg/m^2, respectively.

terns of excretion occurred at all four dosages of carboplatin used in this study.

Due to the schedule of five daily injections employed in this study, the opportunity existed to assess the effect, if any, of repeated dosing of carboplatin on platinum pharmacokinetics. As mentioned above, in only one patient, who had extensive extravascular fluid, was there any accumulation of plasma platinum. In addition, complete plasma and urinary studies were performed in three patients on Days 1 and 4 of treatment. In all cases, the measurements made on Day 4 were similar to those made on Day 1.

The presence of a pleural effusion in one patient in the study provided the opportunity to assess directly the penetration of platinum, delivered iv as carboplatin, into "third-space" fluids. Pleural effusion concentrations of platinum increased rapidly for the first 30 minutes after drug injection and by 2 hours equaled the concentrations of platinum present in plasma and plasma ultrafiltrate (fig 7).

DISCUSSION

The results of this clinical phase I study indicate that carboplatin is a well-tolerated drug with predictable dose-related side effects. The major dose-limiting side effect is dose-related thrombocytopenia. For solid tumor phase II studies, a dose of 77 mg/m^2/day × 5 is recommended for patients who have received prior chemotherapy. In similar studies, patients with no prior chemotherapy experience should receive 99 mg/m^2/day × 5. We did not escalate doses > 99 mg/m^2/day, since at this level, three of six patients previously untreated with chemotherapy developed platelet count nadirs < 90,000/µl.

TABLE 4.—Pharmacokinetic parameters of total platinum in plasma of patients treated with carboplatin

Dose (mg/m^2)	A ($\mu g/ml$)	α (min^{-1})	α-half-life (mins)	B ($\mu g/ml$)	β (min^{-1})	β-half-life (mins)	AUC ($\mu g/min/ml$)	CL_{TB} ($ml/min/m^2$)	Vd_{SS} (L/m^2)
11*	0.72	0.025	28	0.40	0.0012	567	394	15	22.6
55†	3.55	0.026	26	0.87	0.0014	492	745	39	43.2
77‡	5.98	0.014	49	0.45	0.0006	1188	1358	30	69.6

* Values derived from 4 courses given to 3 patients.
†Values derived from 3 courses given to 3 patients.
‡Values derived from 3 courses given to 3 patients.

TABLE 5.—Pharmacokinetic parameters for ultrafilterable platinum in plasma of patients treated with carboplatin

Dose (mg/m^2)	A ($\mu g/ml$)	α (min^{-1})	α-half-life (mins)	B ($\mu g/ml$)	β (min^{-1})	β-half-life (mins)	AUC ($\mu g/min/ml$)	CL_{TB} ($ml/min/m^2$)	Vd_{SS} (L/m^2)
11*	0.571	0.057	12.2	0.40	0.0029	236	145	40	23.7
22†	1.80	0.091	7.6	1.01	0.0048	146	235	49	17.7
55‡	2.14	0.032	21.4	1.22	0.0052	133	290	100	24.0
77§	4.20	0.068	10.2	4.17	0.0068	102	709	57	16.1

* Values derived from 3 courses given to 3 patients.
†Values derived from 3 courses given to 2 patients.
‡Values derived from 3 courses given to 3 patients.
§Values derived from 3 courses given to 3 patients.

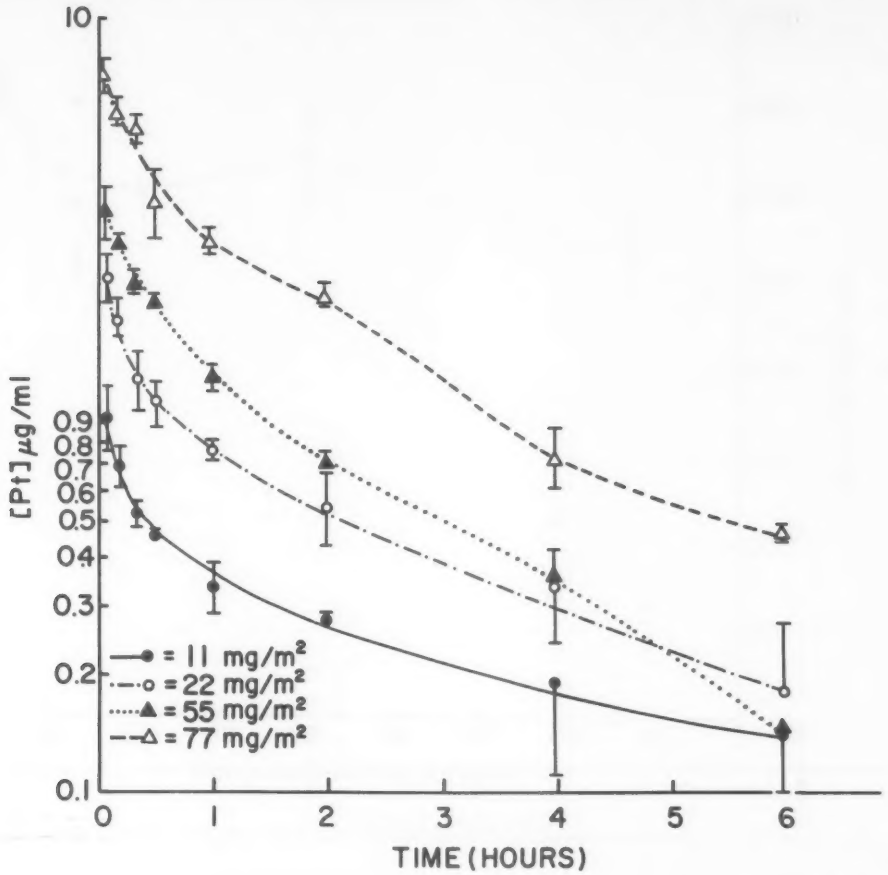

FIGURE 3.—Concentrations of ultrafilterable platinum in plasma of patients treated with carboplatin. Carboplatin was injected as an iv bolus, plasma ultrafiltrates obtained with Amicon Centriflo CF50A cones, and ultrafilterable platinum determined by flameless atomic absorption spectrometry. Points represent means ± SE of 4, 3, 3, and 5 courses given to 3, 2, 3, and 3 patients at doses of 11, 22, 55, and 77 mg/m^2, respectively.

Courses of carboplatin given on an every day × 5 schedule should be repeated every 5 weeks to avoid the possibility of cumulative wbc count toxicity, since leukopenia frequently did not occur until Day 28. Patients noted few side effects due to carboplatin therapy and they were able to maintain normal nutrition and daily activities. The nausea and vomiting encountered in this carboplatin every day × 5 study was insignificant compared to the nausea and vomiting encountered with *cis*-DDP therapy. The cause of the lower-extremity myalgias observed in two patients is not known. Neither patient had any electrolyte or muscle enzyme abnormalities while receiving carboplatin nor any prior exposure to neuropathic anticancer agents. Neither patient had experienced this symptom before or after carboplatin therapy. In contrast to *cis*-DDP, carboplatin, even at high cumulative doses, did not produce any alteration in hearing, renal function, or serum concentrations of electrolytes such as magnesium. These data are similar to the carboplatin phase I study of Rozencweig et al (12), which also investigated a daily × 5 schedule. The median wbc count nadir day at the 100-mg/m^2/day × 5 dosage level was 37, with recovery on median Day 42. The recommended phase II dose and schedule from that study is 100 mg/m^2/day × 5, with courses repeated every 6 weeks.

The pharmacokinetic behavior of platinum administered as carboplatin is strikingly different from that administered as *cis*-DDP. Although our studies do not define the basis for this disparity, it most likely resides in the difference between the bidentate leaving group present in carboplatin as opposed to the two chlorine atoms present in *cis*-DDP. Our studies do clearly show that, al-

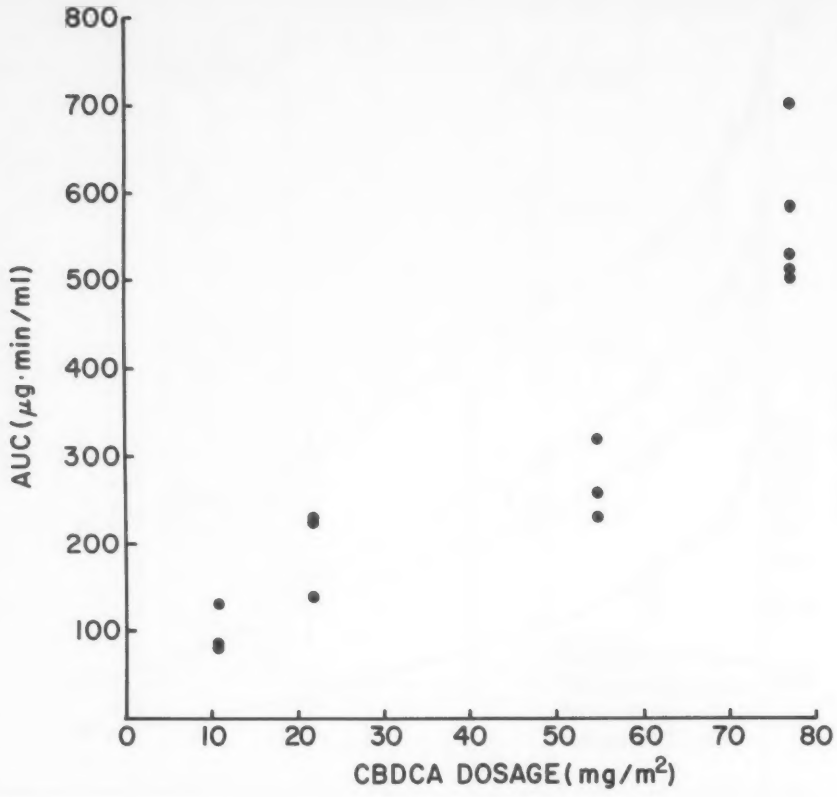

FIGURE 4.—Relationship of AUC of plasma ultrafilterable platinum to dosage in patients treated with carboplatin. Points represent individual courses of carboplatin.

though theoretically possible, carboplatin does not behave as a pro-drug with conversion to *cis*-DDP. This could conceivably occur via rapid dissociation in vivo of the cyclobutane dicarboxylate moiety and replacement with chlorine atoms.

Many of the pharmacokinetic differences between *cis*-DDP and carboplatin reflect differences in binding to plasma proteins. Our results indicate that carboplatin platinum, while binding to plasma proteins, does so much more slowly and possibly less avidly than does platinum administered as *cis*-DDP (13-15). This is shown directly by the fact that most plasma carboplatin platinum is non-protein-bound. In that the short plasma half-life of ultrafilterable *cis*-DDP platinum primarily reflects its avid and covalent binding to plasma proteins, the differences in protein binding between *cis*-DDP and carboplatin are reflected in the much longer plasma half-life of unbound carboplatin platinum. Conversely, the reduced protein binding of carboplatin platinum is reflected in the fact that a much larger percentage of a dose of carboplatin platinum is excreted in the urine than is platinum from *cis*-DDP (13-16). The combinations of these effects are further reflected in the much shorter half-life of total plasma platinum when injected as carboplatin as compared to *cis*-DDP (16,17). Although the differences in binding to plasma proteins by carboplatin platinum and *cis*-DDP platinum might reflect differences in the doses of the two agents that were employed, our results argue against this. The highest dosage of carboplatin employed in our studies (99 mg/m²) is similar to the dosages of *cis*-DDP employed in several studies which examined platinum pharmacokinetics (14-16). In addition, in our study the pharmacokinetics of carboplatin platinum were linear, with no consistent dose-related changes in half-life, CL_{TB}, or Vd_{SS}. More specifically, at all dosages examined, the CL_{TB} of ultrafilterable carboplatin platinum was two to three times the creatinine clearance and the Vd_{SS} of 16.1-24 L/m² approximated the volume of total-body water. Our observation of the linearity of carboplatin platinum pharmacokinetics agrees with the recently

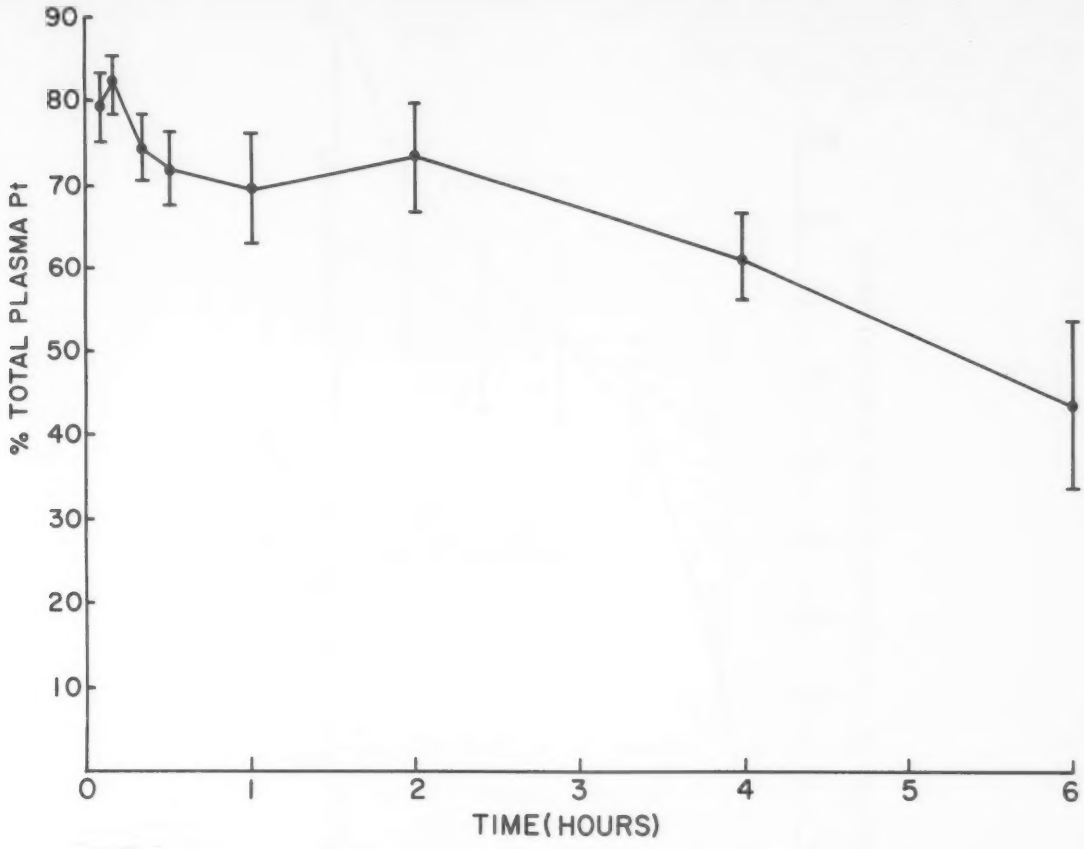

FIGURE 5.—Percentage of plasma platinum that was ultrafilterable. Points represent means ± SE of 15 courses of carboplatin therapy given to 8 patients.

published studies of Calvert et al (9) and implies that the clearance processes for this drug are not saturated by the dosages employed in our study. Our results also agree with those of Calvert et al (9) with regard to the major role played by the kidney in elimination of carboplatin platinum; however, our measurement of the percentage of the dose of platinum excreted in urine in 24 hours is slightly less than the 67% ± 2% reported previously.

Several aspects of our studies deserve further comment. There was no evidence of alteration of carboplatin platinum pharmacokinetics by repeated dosing on Days 1–4 of each course. There is no reason to expect induction of enzymatic metabolism of platinum, since the conversion of *cis*-DDP to other chemical species is felt to reflect physical-chemical rather than enzymatic processes. Moreover, our observations imply that administration of therapeutic doses of carboplatin by repeated small iv boluses is no more nephrotoxic than the administration of a single iv bolus of 400–500 mg/m^2 (9). Other results from our studies raise the issue of whether large accumulations of extravascular fluid such as pleural effusions or ascites can serve as depots for active drug and whether patients possessing such accumulations should receive reduced doses of carboplatin. The two patients in our study with either ascites or pleural effusions received 11 and 55 mg/m^2, respectively, and did not show evidence of enhanced myelosuppression. A similar question has been raised by Calvert et al (9) with regard to patients with renal failure. In that all patients in our study had creatinine clearances > 60 ml/minute, we were unable to evaluate the pharmacokinetics and toxic effects in patients with reduced renal function. However, we are now actively pursuing this question by extending our studies to patients with compromised renal function.

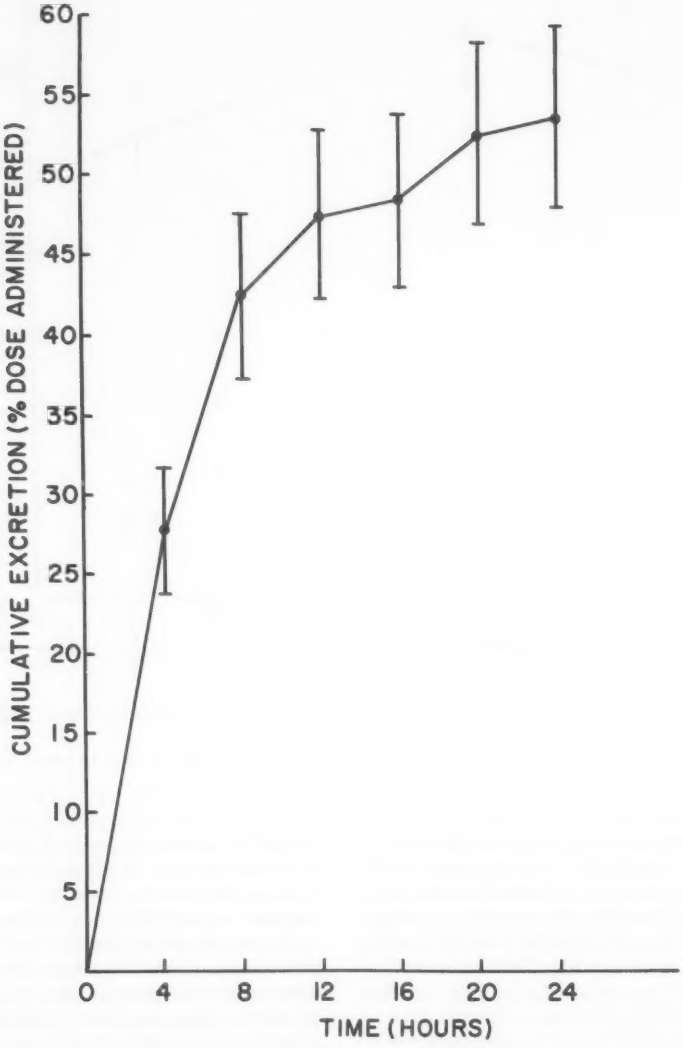

FIGURE 6.—Urinary excretion of platinum by patients injected with carboplatin. All urine voided by patients was collected and stored as pooled 4-hr aliquots. Platinum content was determined by flameless atomic absorption spectrometry. Points represent means ± SE of 11 courses of carboplatin therapy given to 8 patients.

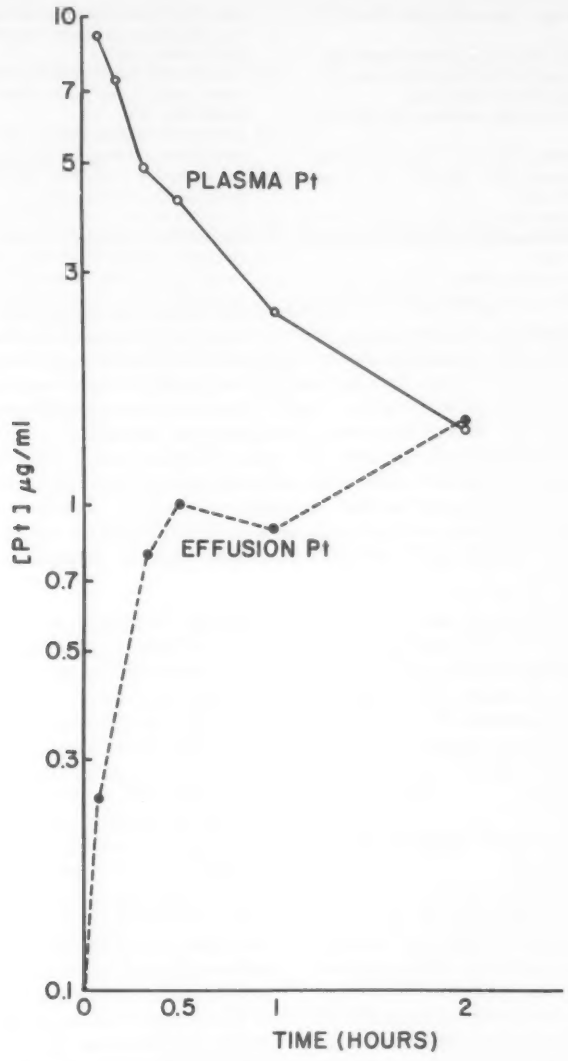

FIGURE 7.—Concentrations of platinum in the plasma and pleural effusion of a patient injected with 77 mg/m^2 of carboplatin. Samples of plasma, plasma ultrafiltrate, and pleural effusion were obtained at times indicated and platinum content determined by flameless atomic absorption spectrometry.

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Phase I Trial of Teroxirone[1,2]

James A. Neidhart,* Diane Derocher, Michael R. Grever, Eric H. Kraut, and Louis Malspeis[3,4]

Teroxirone is a novel triepoxide, synthesized as an alkylator and showing a broad spectrum of preclinical activity. It has good cytotoxic activity against sublines of P388 and L1210 leukemias resistant to another alkylating agent, cyclophosphamide. Thirty-six patients received teroxirone as a single iv push for 5 sequential days every 4 weeks. The dose-limiting toxic effects were phlebitis and cutaneous "flare" reactions, with a maximal tolerated dose of 340 mg/m²/day × 5. Nausea, vomiting, and myelosuppression were present but were not dose-limiting at the maximal tolerated dose. This dose would probably be a reasonable dose for phase II trials, but could not be delivered repeatedly without a central line. Since the local reactions were very severe and unique, we believe that studies on the safety of repeated administration via a central line should be completed in animals before trials of systemic therapy in humans begin. [Cancer Treat Rep 68:1115–1119, 1984]

Teroxirone (alpha-TGT) is a triepoxide derivative originally synthesized by Budnowski, Henkel Cie, Frankfurt, West Germany (1). Synonyms include Henkel's compound and triazine triepoxide. Both the alkylating capacity (2) and the cytotoxic activity of bifunctional epoxides have been known for several years (3,4). Alpha-TGT is a novel compound containing three epoxy groups and is a mixture of two stereoisomers. The alpha isomer was selected for clinical trials due to its greater activity (5,6), solubility (6), and lesser toxicity (5). Alpha-TGT showed good antitumor activity against murine leukemias and solid tumors when both tumor and drug were administered ip. Activity against tumors implanted sc (6) or im (5,7) was present but was generally less impressive than that against tumors implanted ip. Activity is schedule-dependent. The daily 5- or 9-day ip schedules show up to tenfold the activity against L1210 leukemia of an equivalent dose given as a single injection (5,6). Alpha-TGT was also shown to have good activity against a P388 line refractory to the alkylating agent, cyclophosphamide (5). Lethal toxicity in mice included weight loss, diarrhea, nervousness, adipsia, and epidermal changes. A dose equivalent to that producing death in 10% of mice (LD10) was well-tolerated by dogs on the 5-day dosing regimen. Twice that dose produced reversible CNS, marrow, and gastrointestinal toxic effects and a decrease in physical activity. Necrosis and inflammation were seen at sites of injection in the guinea pig and rabbit (6). The study reported here was designed to determine the optimal dose and dose-limiting toxic effects in humans with alpha-TGT given as a single injection for 5 consecutive days every 4 weeks.

STUDY DESIGN AND PATIENT SELECTION

The starting dose was one-tenth the murine LD10 or 16 mg/m²/day for 5 sequential days given as a 10-minute iv infusion. Treatments were repeated every 4 weeks if patients had fully recovered from toxic effects of the previous course. Doses were subsequently escalated by a modified Fibonacci scheme as shown in table 1. Dose escalations were permitted in individual patients who had received three courses of alpha-TGT without significant toxicity. Toxic effects were graded using World Health Organization criteria with minor modifications. Basically, grade 1 toxicity did not significantly interfere with function and did not require therapy; grade 2 required treatment and interfered with function; grade 3 was in-

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TABLE 1.—Hematologic toxicity

Dose* (mg/m^2/day)	No. of evaluable patients/ No. of courses	Leukocyte count ($\times 10^{-3}$ cells/mm^3) Nadir range	Median nadir (Day)	No. of patients suppressed†	Platelet count ($\times 10^{-3}$ cells/mm^3) Nadir range	Median nadir (Day)	No. of patients suppressed†
16	3/2	5.0-9.4	7.2 (21)	0	169.0-212.0	190.5 (30)	0
32	3/4	4.9-5.7	5.45 (16)	0	120.0-202.0	162 (16)	0
52	4/6	2.4-6.5	4.3 (17.5)	1	103.0-495.0	235 (21)	0
90	4/4	2.7-6.2	3.35 (19)	1	34.0-365.0	180.5 (21.5)	1
112	3/4	4.0-12.3	6.4 (21)	0	33.0-200.0	186.0 (31)	1
144	3/3	3.8-17.0	7.1 (10)	0	61.0-165.0	255.0 (20)	1
192	4/7	2.9-5.2	4.3 (20)	1	171.0-541.0	285.0 (16)	0
256	15/28	0.5-14.3	3.35 (20)	4	5.0-476.0	184.0 (18)	4
340	10/15	0.9-9.6	3.2 (21)	6	23.0-364.0	166.0 (19)	4

* Iv push on Days 1-5.
†Wbc count < 3000 or platelet count < 100,000/mm^3.

tolerable and required an alteration or cessation of anticancer treatment; and grade 4 was life-threatening.

Alpha-TGT was supplied by the Investigational Drug Branch, Division of Cancer Treatment, Cancer Therapy Evaluation Program, National Cancer Institute (Bethesda, MD) as 100 mg of white lyophilized powder with 200 mg of mannitol in a 20-ml vial to be reconstituted with sterile water. The final pH of the solution is 6.0-8.0 and the reconstituted drug is stable for at least 4 hours at room temperature (6).

Cbcs were obtained weekly throughout the study. Liver enzymes (SGOT and SGPT), LDH, alkaline phosphatase, glucose, electrolytes, BUN, creatinine, urinalysis, prothrombin time, and partial thromboblastin time were obtained prior to the first and second courses of therapy and when the patient was removed from the study. They were repeated with each course if alpha-TGT-induced abnormalities were noted.

Patients with histologically proven cancer refractory to known effective treatment who gave informed consent were eligible for entry in the study. Patients had either a functional capacity of 2 or better or a life expectancy of at least 12 weeks; normal liver, renal, and bone marrow functions; and full recovery from any prior therapies. Exceptions to these guidelines required approval by the principal investigator and were rare (table 2).

RESULTS

Thirty-six patients were entered in this study between April 7, 1982, and December 1, 1982, and all had completed therapy as of March 1983. Eighty-two courses were given with up to seven courses per patient. Table 2 shows demographic data. Thirty-four patients are evaluable for hematologic toxicity and all are evaluable for nonhematologic toxicity. Most patients (69%) had a good functional capacity. The other patients usually had either pain limiting their function or very slowly progressive disease, and it was thought that they would be able to complete a full course of therapy. Ten patients had received no prior therapy. An additional 14 patients had received only one prior therapy, and the remaining 12 had been heavily pretreated.

Hematologic toxicity is shown in table 1. A myelosuppressive effect of alpha-TGT was seen at both the 256- and the 340-mg/m^2/day levels, although dose-limiting myelotoxicity was not seen at either level. Seven of the 15 patients receiving 256 mg/m^2/day and seven of the 11 receiving 340 mg/m^2/day had a wbc count nadir < 3000/mm^3, a platelet count nadir < 100,000/mm^3, or both. The incidence and degree of leukopenia and thrombocytopenia were similar. At these doses an occasional patient developed marked myelosuppression. Cumulative hematologic toxicity was not seen. Nadirs were seen ap-

TABLE 2.—Patient characteristics

Characteristic	No. of patients (%)
Total	36
Evaluable for hematologic toxicity	34
Evaluable for nonhematologic toxicity	36
Median age in yrs (range): 55 (18-81)	
Performance status (Zubrod)	
0-1	13 (36)
2	12 (33)
3	10 (28)
4	1 (3)
Sex	
Male	21
Female	15
Prior therapy	
No prior chemotherapy	10
Prior chemotherapy	26
Median No. of prior regimens (range): 1 (1-3)	
Median No. of prior agents (range): 3 (1-7)	
Prior radiation therapy	23
Diagnosis	
Colorectal	10 (28)
Lung	7 (19)
Head and neck	5 (14)
Renal	3 (8)
Miscellaneous	11 (31)

proximately 18–21 days after initiation of treatment and were reversible within 4 weeks.

Nonhematologic toxicity is shown in table 3. The dose-limiting toxic effect of alpha-TGT is an unusual and often severe local reaction that is more severe than the phlebitis seen with other vesicant anticancer agents. Figure 1 shows the veins of a patient who developed a severe bullous reaction that eventually ulcerated. Even patients who did not develop the bullous phlebitis had marked discoloration and sclerosis of the veins which necessitated a central line for treatment after several courses. The cutaneous "flare" reactions were often hard to separate from the phlebitis except that they were more localized and formed painful indurated circular areas at or proximal to sites of infusion. We do not believe these were the result of extravasation in any patient. Reactions tended to be delayed in onset, sometimes beginning several days after completion of treatment and peaking over the next several weeks. Neither steroids, antihistamines, dilution, nor prolonging infusion times up to 6 hours prevented these reactions. These reactions occurred in six of the 11 patients at the 340-mg/m²/day level and required Hickman catheters for patients receiving multiple courses. Eight of the 15 patients receiving 240 mg/m²/day had reactions although they were usually less severe. Nausea and vomiting were major problems at the two highest dosing levels. Other side effects were usually tolerable.

Studies of the plasma pharmacokinetics of alpha-TGT were undertaken in five patients. Plasma levels were determined employing a high-performance liquid chromatographic assay in which the drug was derivatized with sodium piperidine dithiocarbamate, the derivative extracted with benezene, and the solvent removed by evaporation prior to chromatography. The extract was reconstituted in methylene chloride and chromatographed on a 5-μm silica column using a mobile phase of hexane:methylene chloride:methanol (20:6.7:1). Selective detection of the derivative was achieved at 254 nm. The method allows assay of concentrations down to 25 ng of alpha-TGT in 1 ml of plasma, with a relative standard deviation of 7.5%. The plasma concentration-time profiles of the patients following the short iv infusion of alpha-TGT exhibited a rapid monoexponetial decline. Plasma pharmacokinetic parameters (table 4) were calculated employing a one-compartment open model with a zero-order input function for the injection period. Alpha-TGT is very rapidly cleared from plasma with a harmonic mean half-life of 0.91 minutes and a mean total-body plasma clearance of 1.12 L/minute/m² in the five patients.

Thirteen patients in this phase I trial could be evaluated for response by virtue of having measurable disease, optimal dosing (≥ 256 mg/m²/day), and having completed two full courses of therapy. No responses were seen. Three patients had colorectal cancer, four had refractory lung cancer, and two had refractory epidermoid cancer of the oropharynx.

DISCUSSION

The dose-limiting toxicity of alpha-TGT on a Day-5 dosing regimen using infusion by peripheral veins is a unique local reaction consisting of severe phlebitis and/or cutaneous flare. Doses producing the reaction do cause moderate, but not dose-limiting, myelosuppression and probably are near the optimal dose for phase II testing. Administration of this agent on a prolonged basis by peripheral veins would be difficult if not impossible. A central line with or without a slower infusion rate might make prolonged therapy feasible but would, in our opinion, need to be preceded by careful studies in animals demonstrating safety of a central infusion for prolonged periods. The stability of alpha-TGT might also require more careful evaluation if extended infusion times are used. Phlebitis was reported in other phase I trials although usually at a dose higher than that reported here (6,8,9). Piccart et al (9) reached a maximal tolerated dose of 2000 mg/m², with phlebitis as the dose-limiting toxic effect. Their schedule was similar to ours and marrow

TABLE 3.—Nonhematologic toxic effects

Dose (mg/m²/day)	No. of evaluable courses	Toxic courses (total/grade > 2)				
		Vomiting	Phlebitis	Cutaneous*	Alopecia	Miscellaneous†
16	3	0	0	0	0	Hypertension (1)
32	4	0	0	0	0	
52	6	0	0	0	0	
90	5	1/0	1/1	0	0	Diarrhea (1)
112	5	1/0	0	0	0	
144	3	0	0	0	0	
192	9	2/1	2/2	3/1	0	
256	28	9/7	7/1	3/0	2	Diarrhea (1), fatigue (3), nervousness (1)
340	17	2/2	9/7	1/0	1	

* Arm pain, burn on injection, local flare.
†Values in parentheses = No. of patients.

FIGURE 1.—Ulcerative phlebitis and eschar formation 26 days after infusion.

suppression was less in their study than in ours. The formulation supplied by the National Cancer Institute and used in our study is different from that used in the European trials and might account for this discrepancy in the maximal tolerated dose.

The rapid clearance of alpha-TGT from plasma suggests a one-pass effect, the mechanism of which has not, to our knowledge, been defined. Spreafico et al (7) mentioned the short half-life and speculated on the existence of an active metabolite although no data were provided. Certainly, the rapid clearance did not prevent systemic effects in our study since patients developed myelosuppression, nausea, vomiting, and alopecia as well as local reactions. The metabolism, mode of clearance, and mechanism of action of this agent remain largely speculative. Its structure suggests that it alkylates but there are no published data to confirm such an activity or to relate alkylation to the cytotoxic effect of alpha-TGT. The substantial activity of alpha-TGT against tumors refractory to standard alkylating agents has important clinical connotations. Knowledge in this area might facilitate and optimize development of alpha-TGT or related agents.

Alpha-TGT is a unique compound with a broad spectrum of preclinical activity and remains an interesting

TABLE 4.—Pharmacokinetics

Patient No.	Infusion time (mins)	Half-life (mins)	Vd (L/m^2)	Cl_T ($L/min/m^2$)
1	5.1	0.97	2.23	1.60
2	12.4	1.00	0.92	0.64
3	10.7	0.72	0.72	0.69
4	24.4	0.76	0.63	0.57
5	9.7	1.33	4.03	2.10
Mean		0.91*	1.71	1.12
SD		—	1.45	0.69

* Harmonic mean.

and promising agent. We do not believe, unfortunately, that it can be safely administered under the conditions of this study at myelosuppressive doses. Exploration of different dosing regimens and/or routes of administration will be necessary before this agent can enter phase II trials. Intracavitary therapy with alpha-TGT might be one reasonable alternative since it was both safe and effective by this route in preclinical studies.

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Phase II Study of Acivicin in Colorectal Carcinoma: A National Cancer Institute of Canada Study[1]

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Twenty previously untreated patients with advanced colorectal carcinoma were treated with acivicin given in a 5-day iv schedule at a starting dose of 15 mg/m², escalating to 26 mg/m². Therapy was repeated every 3 weeks. Nineteen patients were evaluable for toxicity, which was mild and tolerable overall. Hematological toxicity was minimal, with only one patient developing severe myelosuppression at the starting dose. Nonhematological toxicity was also mild to moderate, occurring in 70% of the patients. Nausea, vomiting, and stomatitis were the most common side effects. CNS toxicity was moderate and occurred in 55% of the patients. Some patients found these symptoms distressing, although the treatment had to be discontinued in only one patient. There were no objective responses among 17 patients evaluable for response. This study indicates that acivicin given in a 5-day schedule is well-tolerated overall. [Cancer Treat Rep 68:1121–1123, 1984]

Acivicin (AT-125) (1) is a new amino acid antibiotic isolated from *Streptomyces sviceus*. Preclinical data demonstrated antitumor activity against a variety of experimental models. These models included L1210 and P388 leukemias, CD8F1 mammary tumor, and human xenograft tumors, particularly the LX-1 lung and MX-1 mammary tumors.

In phase I clinical trials (2–4), gastrointestinal and hematological toxic effects were mild. Central nervous system toxic effects were dose-limiting and consisted of lethargy, confusion, nightmares, paranoid reactions, and jerky movements. The toxic effects developed during treatment and were dose-related and reversible on cessation or reduction of the drug dosage. The exact mechanism of CNS toxicity is not fully elucidated; it may be related to AT-125 close structural resemblance to the known CNS active materials, ibotenic acid (an aspartate agonist), and muscimol (a gamma aminobutyric acid agonist) (5,6). Muscimol, when used in humans as an antischizophrenic agent, had the same CNS side effects as those being observed in AT-125-treated patients (7).

The present phase II study was initiated to determine the efficacy of a 5-day iv schedule in patients with colorectal carcinoma who have not been previously exposed to chemotherapy.

MATERIALS AND METHODS

Patients with histologically confirmed colorectal adenocarcinoma, without prior chemotherapy, were entered in this trial. Eligibility criteria included informed consent; measurable lesions to evaluate antitumor effect; life expectancy of at least 12 weeks with a performance status ≤ 2 according to the Eastern Cooperative Oncology Group criteria; adequate bone marrow function with an absolute granulocyte count of $\geq 2000/mm^3$ and platelet count of $\geq 100{,}000/mm^3$; and adequate hepatic and renal functions with bilirubin ≤ 2.0 mg/100 ml, BUN ≤ 20 mg/100 ml, and serum creatinine ≤ 1.5 mg/100 ml.

On the basis of the phase I data, AT-125 was administered by a rapid iv infusion at a starting dose of 15 mg/m²/day × 5. Treatment was repeated every 21 days if the counts were adequate. An adequate trial for toxicity consisted of one course followed by 3 weeks' observation.

Dose modifications were included in the trials with 20% escalation if the lowest granulocyte count was $> 1500/mm^3$ and if the lowest platelet count was $> 100{,}000/mm^3$ and with a 20% decrease if the lowest granulocyte count was $< 750/mm^3$ and/or the platelet count was $< 75{,}000/mm^3$.

Standard response criteria were used. Complete remis-

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sion was defined as the disappearance of all clinical evidence of disease for a minimum of 4 weeks. Partial remission was defined as a ⩾ 50% decrease in the sum of the products of the diameters of measured lesions with no simultaneous increase or the appearance of new lesions. Progressive disease was defined as an unequivocal increase of at least 25% in the size of any lesion or the appearance of new lesions. Stable disease was defined as a response less than partial remission or progression less than progressive disease without appearance of any new lesions.

RESULTS

Twenty patients (11 males and nine females) were entered in the study. The median age was 59 years (range, 38–77). Three patients were inevaluable for response; they consisted of one patient receiving an incomplete cycle due to toxicity, one patient removed prematurely from the study due to brain metastases, and one patient with early unrelated death (the only patient inevaluable for toxicity). Three patients received prior radiotherapy to sites different than those being evaluated for response. The liver was the most common site of metastatic disease, being involved in 15 patients (75%).

A total of 75 cycles of chemotherapy were administered in this study. From the starting dose of 15 mg/m^2, we were able to escalate the drug to 18 mg/m^2 in nine patients (45%), to 22 mg/m^2 in four patients, and to 26 mg/m^2 in one patient with minimal toxicity, but the treatment was discontinued due to progressive disease.

Hematological toxicity was minimal; only one patient with prior radiotherapy developed thrombocytopenia at the starting dose with a platelet count of 45,000/mm^3 and required dose reduction. The median platelet count nadir was 204,000/mm^3 (range, 45,000–572,000). One patient with a granulocyte count of 1170/mm^3 developed fever which responded well to antibiotic therapy. The median granulocyte count nadir was 2100/mm^3 (range, 700–7300).

Nonhematological toxicity was also mild to moderate. The most common symptoms have been mild to moderate nausea and vomiting; another consistent symptom has been burning of the mouth during the last 2 days of therapy and during subsequent days one patient had definite stomatitis. Other unusual symptoms consisted of odd taste, diarrhea, vaguely localized abdominal pain, and skin desquamation of hands. Only two patients had their treatment discontinued due to side effects; one had generalized weakness and abdominal pain and the other had recurrent nausea and vomiting.

Although CNS toxicity was considered dose-limiting in phase I studies, the drug was well-tolerated in this 5-day schedule. At a dose of 15 mg/m^2, mild to moderate CNS toxicity occurred in eight patients (40%); however, following dosage escalation there was a slight increase in the severity of symptoms as well as in the incidence of CNS symptoms, being observed in 11 patients (55%). CNS symptoms consisted mainly of confusion, depression, sleeping difficulty, sense of unreality, and difficulty in concentration. Subjective unsteadiness, olfactory hallucination, and tremor were reported in a single patient each. In most patients, these symptoms occurred within 5 days of therapy and were always reversible. Although some of these patients found these symptoms quite distressing, only one patient refused further therapy in view of difficulty in sleeping and nightmares after the second cycle of therapy. Details of nonhematological toxicity are shown in table 1.

In 17 patients evaluable for response, no objective response was noted. Nine patients had stable disease, with a median duration of 4 months (range, 1–10). The other eight patients had progressive disease.

DISCUSSION

Our study indicates that AT-125 given in a 5-day schedule is well-tolerated overall. Hematological toxicity was minimal and only one patient who had had prior radiotherapy had to have his initial dose reduced. Nonhematological toxicity was also mild to moderate with gastrointestinal symptoms being the most prominent, in addition to generalized weakness and tiredness. Based on phase I data, neurological toxicity was carefully observed. However, in this schedule it proved to be minimal, with 60% of patients having no toxicity at the starting dose of 15 mg/m^2. In the other patients, side effects were mild and reversible. Nevertheless, some patients found these symptoms quite distressing, but only one patient refused therapy due to a combination of depression, nightmares, and nausea and vomiting. There was a slight increase in the incidence and severity of side effects following dose escalation; nevertheless, we were able to escalate the drug dosage in nine patients (45%), with the highest dose

TABLE 1.—Nonhematological toxicity

Toxic effect	No. of patients
General weakness	7
Nausea/vomiting	12
Stomatitis/burning of mouth	4
Anorexia	2
Odd taste	3
Diarrhea	4
Abdominal pain	2
Skin desquamation	3
Confusion	3
Depression	3
Sleeping difficulty/nightmares	6
Sense of unreality	2
Difficulty in concentration or calculations	2
Subjective unsteadiness	1
Olfactory hallucinations	1
Tremor	1

highest dose in the study reaching 26 mg/m^2 in one patient. No objective responses were documented in this study.

In conclusion, AT-125 given in a 5-day iv schedule is well-tolerated, but given in this schedule it does not appear to be active in colorectal cancer.

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Plasma Kinetics of Oral Retinol in Cancer Patients[1,2]

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Concurrent with a phase I trial of retinol in patients with advanced cancer, we studied the plasma kinetics of both retinol and its major metabolites, retinyl palmitate and retinyl stearate. Retinol was administered to 12 patients in daily oral doses of 60,000, 100,000, 150,000, or 200,000 units/m². Patients remained on treatment until the development of dose-limiting toxic effects or disease progression. Retinoid plasma kinetics were studied on the first day of treatment, at Weeks 2 and 4, and every 2–3 months thereafter as long as the patient remained on therapy. A high-performance liquid chromatography assay was used to quantitate the plasma concentration of both retinol and its fatty acid esters. There was no significant change in the plasma retinol concentration up to 24 hours after a single oral dose of retinol ($P > 0.05$). However, the plasma concentration of retinyl palmitate and retinyl stearate markedly increased with a mean time to peak plasma concentration of 4.3 ± 0.7 hours. Retinyl palmitate rapidly disappeared from the plasma with an initial phase half-life of 2.2 ± 0.9 hours. The terminal phase half-life appeared prolonged and could not be accurately determined. Retinyl stearate was detected in the plasma of all patients with plasma concentrations paralleling and ranging from 20% to 40% those of retinyl palmitate. With prolonged retinol administration, peak plasma retinyl palmitate concentrations increased with both increasing retinol dose ($P < 0.001$) and increasing duration of treatment ($P = 0.001$). In three patients with low pretreatment plasma retinol concentrations, daily retinol administration was associated with a rise in plasma retinol concentration. Because only one patient developed serious toxic effects and all patients had markedly increased plasma concentrations of retinyl esters, no conclusion could be made about the relationship between plasma retinyl ester concentration and retinol toxicity. [Cancer Treat Rep 68:1125–1133, 1984]

Vitamin A (all-*trans*-retinol) and its naturally occurring and synthetic analogs (retinoids) are undergoing investigation as antineoplastic and cancer-prevention agents. In animal models, these compounds can prevent or reverse preneoplastic lesions and inhibit the growth of some established cancers (1–3). However, clinical use of the retinoids in the prevention and treatment of human cancers has been slowed because of their toxicity potential. To separate retinoid toxicity from its "antiproliferative" action, a large number of synthetic retinoids have been developed (4). Of these, only 13-*cis*-retinoic acid and the aromatic retinoid, etretinate, have undergone evaluation in man (3,5–7). At present, all-*trans*-retinol (R), retinyl palmitate (RP; the palmitic acid ester of retinol), and retinyl acetate are the major retinoids available for use in clinical trials. Although all three have been available as dietary supplements for many years, there has been little clinical investigation of their pharmacokinetics or anticancer activity (8,9). There have been no prospective clinical trials relating clinical toxicity to plasma retinoid concentration. Toxicity studies in general have been retrospective and consist of literature reviews and case reports of accidental or intentional overdoses (10–13). Prior to the use of retinol in

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TABLE 1.—Clinical characteristics of 12 patients undergoing plasma kinetic evaluation of oral retinol

Patient No.	Dose (units/m²)	Age in yrs	Sex	Treatment duration (mos)	Diagnosis
1	100,000	67	M	5	Adenocarcinoma, colon, liver metastasis
2	100,000	63	F	2	Squamous carcinoma, rectum, local recurrence
3	150,000	67	F	4	Adenocarcinoma, colon, local recurrence
4	150,000	66	F	2	Melanoma, metastatic
5	150,000	56	M	6	Squamous carcinoma, oropharynx (adjuvant)
6	200,000	73	M	9	Adenocarcinoma, colon, Duke's C (adjuvant)
7	200,000	71	M	12	Squamous carcinoma, nasopharynx (adjuvant)
8	200,000	76	M	3	Adenocarcinoma, prostate, stage D
9	60,000	52	M	18	Melanoma (adjuvant)
10	200,000	49	F	2	Adenocarcinoma, ovary, stage III
11	200,000	75	M	4	Basal cell carcinoma, skin, multiple
12	200,000	66	F	1	Adenocarcinoma, endometrium, stage IV

phase II clinical trials for the treatment of advanced cancers, we carried out a phase I study of oral retinol with daily dosing over a 1-year period. The clinical and toxicological findings of this trial have been previously published (14). We are now reporting the results of a prospective evaluation of the plasma kinetics of retinol and its ester metabolites in cancer patients entered in this phase I trial.

MATERIALS AND METHODS

Between November 1979 and January 1982, eight patients were entered in a phase I trial of R. Five patients had advanced cancer and had failed standard therapies, while three had a high risk of recurrence after primary surgery and/or radiation therapy. The clinical characteristics and doses administered to these eight patients are shown in table 1. Informed consent was obtained prior to drug treatment in accordance with the rules of our institutional review committee.

R (Aquasol A, USV Pharmaceutical Corp) was administered orally, once daily, at a dose of 100,000, 150,000, or 200,000 units/m².[5] Three patients each received 200,000 or 150,000 units/m² and two patients received 100,000 units/m². Dose escalation > 200,000 units/m² was not attempted because of previously documented hepatic and CNS toxic effects at a dose of approximately 300,000 units/m² (14).

To examine both acute and chronic plasma kinetics, patients were maintained on daily treatment for 1 year, until disease progression or the development of dose-limiting toxic effects. Kinetic studies were done on the first day of treatment, Day 14, 4 weeks, 8 weeks, and approximately every 2-3 months thereafter until discontinuing therapy. Patients were evaluated for toxic and anticancer effect every 4 weeks. Prior to and during R supplementation, patients were instructed to take no other vitamin supplements and to continue their normal diet.

[5] 1 IU = 0.3 μg of retinol.

Four additional patients receiving R but not on the phase I trial were studied once only with prolonged blood sampling up to 120 hours after the oral administration of 60,000 units/m² (one patient) and 200,000 units/m² (three patients).

On the day of blood sampling after an overnight fast, capsules containing 50,000 and 10,000 units of R were administered at 8:00 AM as an oral bolus. At 60-120-minute intervals, 7-10 ml of blood was collected in foil-wrapped vacutainers containing 100 units of sodium heparin. Samples were stored on ice in the dark prior to centrifugation at 2000 rpm for 10 minutes. The plasma layer was removed, placed in polypropylene freezer tubes (Vangard International Inc, Neptune, NJ), and stored in the dark at -25°C until analysis. Patients were allowed to resume normal oral intake 3-4 hours after drug administration.

Retinoid Assay Procedure

At the time of analysis, the plasma samples were allowed to thaw in the dark at room temperature (23°-28°C). Two hundred microliters of 5% $HC10_4$ were added to a 1.0-ml aliquot of plasma in a glass centrifuge tube and rapidly mixed. A 1.0-ml aliquot of ethyl acetate (glass distilled, Burdick and Jackson, Muskegon, MI) was added, and the samples were mixed again for 60-90 seconds and centrifuged at 5000 g for 5 minutes. Fifty microliters of the resulting organic layer were analyzed by high-performance liquid chromatography (HPLC).

HPLC analysis was performed using two Waters Associates (Milford, MA) Series M-45 solvent delivery systems and a Model 440A UV detector. R was analyzed by using a previously published method (5). To resolve R from the fatty acid esters, RP, and retinyl stearate (RS), an Altec Ultrasphere ODS 5-μm column was used for analysis. The mobile phase consisted of 83% filtered, degassed, and premixed tetrahydrofuran (eight parts), isopropanol (one part), acetonitrile (one part) (Burdick and Jackson, glass distilled), and 17% aqueous ammonium acetate (1%)

(Fisher, Fair Lawn, NJ). The flow rate was 1.5 ml/minute. Quantitation of R and RP was done by an external standard method. Since RS standard was not available, plasma concentrations could not be determined. However, RS peak heights were compared and expressed as a percent of RP.

Recovery and Precision

RP or R was added to 1.0 ml of pooled human plasma yielding final retinoid concentrations ranging from 100 ng/ml to 100 μg/ml. Recovery was calculated by comparing the peak height or peak area of added retinoid to that of retinoid standards. All experiments were carried out in triplicate. Precision was determined by triplicate extraction and assay of three different patient samples.

Retinol Capsular Contents

To quantitatively and qualitatively determine the content of the R capsules, three 10,000- and three 50,000-unit capsules were each dissolved in 100 ml of ethyl acetate. Ten microliters were analyzed by direct HPLC injection and peak heights were compared to a standard curve.

Data Analysis

Plasma concentration of R and RP versus time data was obtained from each study and fitted to a multiexponential equation using a nonlinear regression computer program (15). Preliminary parameter estimates were obtained with the aid of a previously published computer method (16). Equations used in the analysis are identical to those previously published (17). Comparisons were made using the Wilcoxon and Kruskal-Wallis tests (18).

RESULTS

Our HPLC system resulted in a clear separation of R, RP, and RS. Figure 1 shows chromatograms of the fol-

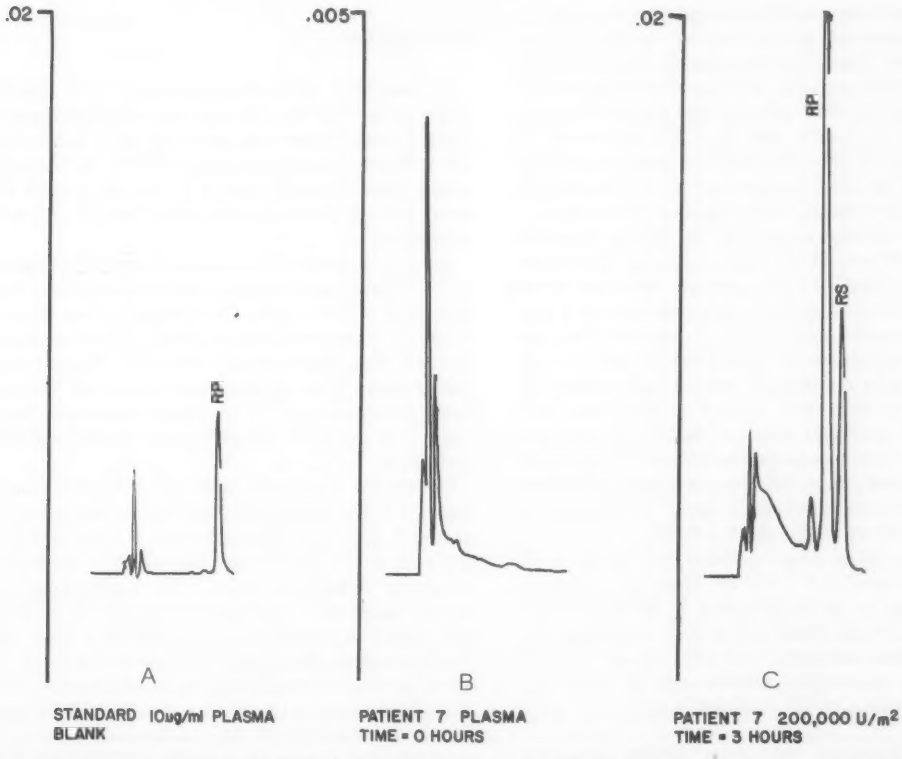

FIGURE 1.—Chromatograms of standard retinoid in plasma and patient samples: A—10 μg/ml of RP added to a sample of pooled plasma (blank); B—extract of Patient 7 plasma prior to oral R (time = 0 hrs); and C—extract of Patient 7 plasma 3 hrs after 200,000 units/m^2 of oral R. Peaks marked RP and RS represent retinyl palmitate and retinyl stearate, respectively.

lowing: an extract of pooled plasma with added (A); an extract of plasma from Patient 7 before oral R administration (B); and Patient 7 3 hours after oral R (200,000 units/m^2) (C).

The recovery of RP from plasma was linear from 100 ng/ml to 100 μg/ml and averaged 97.7% ± 2.9%. The assay had a detection limit of 50 ng/ml determined on the basis of a signal equal to twice the noise level. The precision of the assay for RP from three different patient samples over a tenfold range in concentration averaged 4.4% ± 2.4%.

The HPLC peaks corresponding to RP and RS were analyzed by gas chromatography/mass spectrometry. The collected HPLC peaks revealed major mass ions identical to those of RP standard and those predicted for RS.

Analysis of the R capsules showed that both the 10,000- and 50,000-unit capsules were > 99.9% R; *cis-trans*-retinoic acid, RP, or RS was not detected.

After the oral administration of R in eight patients (29 studies), there were no significant individual acute changes in the plasma R concentration ($P > 0.05$). Since there was no significant dose-response curve after the acute administration of R, a daily average plasma R concentration was determined by dividing the area under the R plasma concentration/time curve by the time interval used (12 hours). These daily averages are listed in table 2. With prolonged treatment, three patients (Patients 4–6) had an increase in their daily average plasma R concentrations, but in all eight patients, there was no significant change ($P = 0.09$) (table 2). There was no significant difference in the daily average plasma R concentration between the three dosage levels examined ($P = 0.22$)

After oral administration of R, the plasma concentration of both RP and RS increased markedly. The plasma kinetics of RP obtained from nonlinear regression fitting of the plasma concentration versus time data to a two-compartment model are summarized in table 2. The average time to peak plasma RP concentration was 4.3 ± 0.7 hours in the eight patients who were serially studied. Although few patients were studied at each dose level, there was no significant difference in time to peak plasma RP concentration between the different R dose levels ($P = 0.590$). Peak plasma RP concentrations significantly increased with both increasing R dose ($P < 0.001$) and increasing duration of treatment ($P = 0.001$).

The average initial phase half-life ($t_{½}$) for RP in all eight patients was 2.2 ± 0.9 hours. There was no significant difference in the initial phase $t_{½}$ during an individual patient's serial studies ($P = 0.27$) or between the three dose levels examined ($P = 0.15$). Because R was administered to these eight patients every 24 hours, the elimination phase $t_{½}$ of RP could not be accurately determined.

Figure 2 illustrates the serial plasma concentration/time curves for a patient receiving 100,000 units/m^2/day of R over a 140-day period. Note that on the first day of R administration, as opposed to most patients, there was an acute change in plasma R concentration. Plasma RP concentrations also increased from undetectable to a peak of 3.9 μg/ml. Over the 140-day study period, R concentrations showed little change, while peak RP levels increased to a maximum concentration of 12.5 μg/ml.

Because of RP's apparent prolonged elimination phase $t_{½}$, four additional patients (Patients 9–12) receiving R were studied with serial blood sampling up to 120 hours after a single oral dose. Additional R was withheld for the duration of these studies. Plasma concentration/time curves for Patients 10–12, who all received 200,000 units/m^2, are illustrated in figure 3. In two patients, the elimination phase $t_{½}$s were 42 hours (Patient 12) and 133 hours (Patient 9). In the other patients (Patients 10 and 11), the elimination $t_{½}$s were markedly prolonged and could not be accurately determined.

RS was detected in the plasma of all patients. Its concentrations paralleled those of RP, but could not be accurately quantitated because of lack of a standard. Peak areas ranged from 20% to 40% of the accompanying RP peak.

DISCUSSION

In designing both chemoprevention and treatment trials using retinoids, it is important to understand both their plasma kinetics and the relationship between plasma retinoid concentration and toxicity. In conjunction with a phase I toxicity trial of R, we used a rapid HPLC assay to study the plasma kinetics of both R and its fatty acid esters.

Evidence suggests that R is absorbed at the small bowel mucosal lining and is rapidly esterified with the intracellular pool of fatty acids. Once formed, these fatty acid esters are transported first via the lymphatic system and then via the systemic blood to the liver. Hepatic storage occurs primarily as the fatty acid esters (19). The subsequent de-esterification of the retinyl esters and their release as R bound to retinol-binding protein are poorly understood.

Our results, along with those of Goodman et al (8) and Huang and Goodman (20), show that despite a high dose of oral R, small-bowel esterification is not rate-limiting, and little unesterified R appears acutely in the lymph or peripheral circulation. After 1–12 months' administration of high-dose R, eight patients did not have a significant change in plasma R concentration ($P = 0.22$). These findings suggest that plasma R concentration is not influenced by either excess R intake or excess hepatic stores of the retinyl esters. In the absence of vitamin A deficiency, plasma R concentration may be determined by hepatic synthesis and release of retinol-binding protein rather than by the amount of dietary or stored retinoid.

Following both acute and chronic R administration, the

plasma concentration of RP and RS markedly increased. In one patient, RP plasma concentrations rose from undetectable (< 50 ng/ml) to > 100 μg/ml. The mean time to peak plasma RP concentrations was 4.3 ± 0.7 hours and was independent of dose administered. These findings agree with studies reported by Goodman et al (8) and Huang and Goodman (20). These investigators administered oral ^{14}C-retinol to animals and human volunteers

TABLE 2.—Serial plasmakinetic parameters of RP after daily administration

Patient No.	Dose (units/m^3)	Day of study	Pretreatment R concentration (ng/ml)	Average R concentration (ng/ml)*	Time to peak RP concentration (hrs)	Peak RP concentration (μg/ml)	RP initial phase $t_{1/2}$ (hrs)
1	100,000	1	472	601.0	3	3.9	1.4
		11		771.1	3	5.2	1.5
		140		903.7	3	12.5	1.0
Mean ± SD of 3 studies				738 ± 151	3		1.3 ± 0.25
2	100,000	1	490	518.1	6	6.4	2.0†
		18		311.6	4	3.2	1.5†
Mean of 2 studies				414.8	5		1.7
3	150,000	1	612	616.1	5	9.1	Unable‡
		16		547.5	4	19.3	5.2
		37		738.2	6	9.8	3.3
		91		603.3	5	21.2	4.7
Mean ± SD of 4 studies				626 ± 80	5 ± .81		4.4 ± 0.9
4	150,000	1	206	210.9	3	5.2	2.1†
		11		268.1	5	10.2	1.5†
		34		354.8	5	14.0	2.0†
Mean ± SD of 3 studies				278 ± 72	4.3 ± 1.15		1.8 ± 3.2
5	150,000	1	61	87.7	5	3.2	1.4
		13		182.1	4	4.4	1.4
		28		376.6	3	10.5	4.1
		56		426.0	4	12.1	1.7
		84		418.2	3	6.1	2.5†
		175		321.0	3	6.8	Unable‡
Mean ± SD of 6 studies				302 ± 138	3.6 ± .81		2.2 ± 1.0
6	200,000	1	232	396.8	5	19.5	1.6
		13		718.2	5	18.2	2.2
		30		752.5	5	18.2	1.4
		85		822.6	5	29.0	Unable‡
		176		912.8	4	36.0	Unable‡
Mean ± SD of 5 studies				721 ± 195	4.8 ± .45		1.7 ± .4
7	200,000	1	662	614.2	4	35	1.3
		13		806.3	4	56	Unable‡
		27		367.4	6	34	3.0
		55		736.0	6	68	Unable‡
		83		570.1	4	81	Unable‡
		181		762.2	5	117	Unable‡
Mean ± SD of 6 studies				643 ± 162	4.8 ± 1		2.1
8	200,000	1	525	543.3	4	16.2	2.6
		14		494.1	5	18.8	2.4
		28		465.2	4	22.5	2.7
		91		470.8	4	29.0	2.4
Mean ± SD of 4 studies				493 ± 35	4.25 ± .5		2.5 ± 0.2
Mean ± SD of all 8 patients					4.3 ± 0.7		2.2 ± 0.9

* Values represent a daily average determined by dividing the area under the plasma concentration curve by the time interval used (12 hrs).
†Plasma concentration/time data could not be accurately fitted to computer-plotted curve. Values listed are estimates.
‡The initial part of plasma concentration/time curve was flat. Initial phase $t_{1/2}$ could not be estimated.

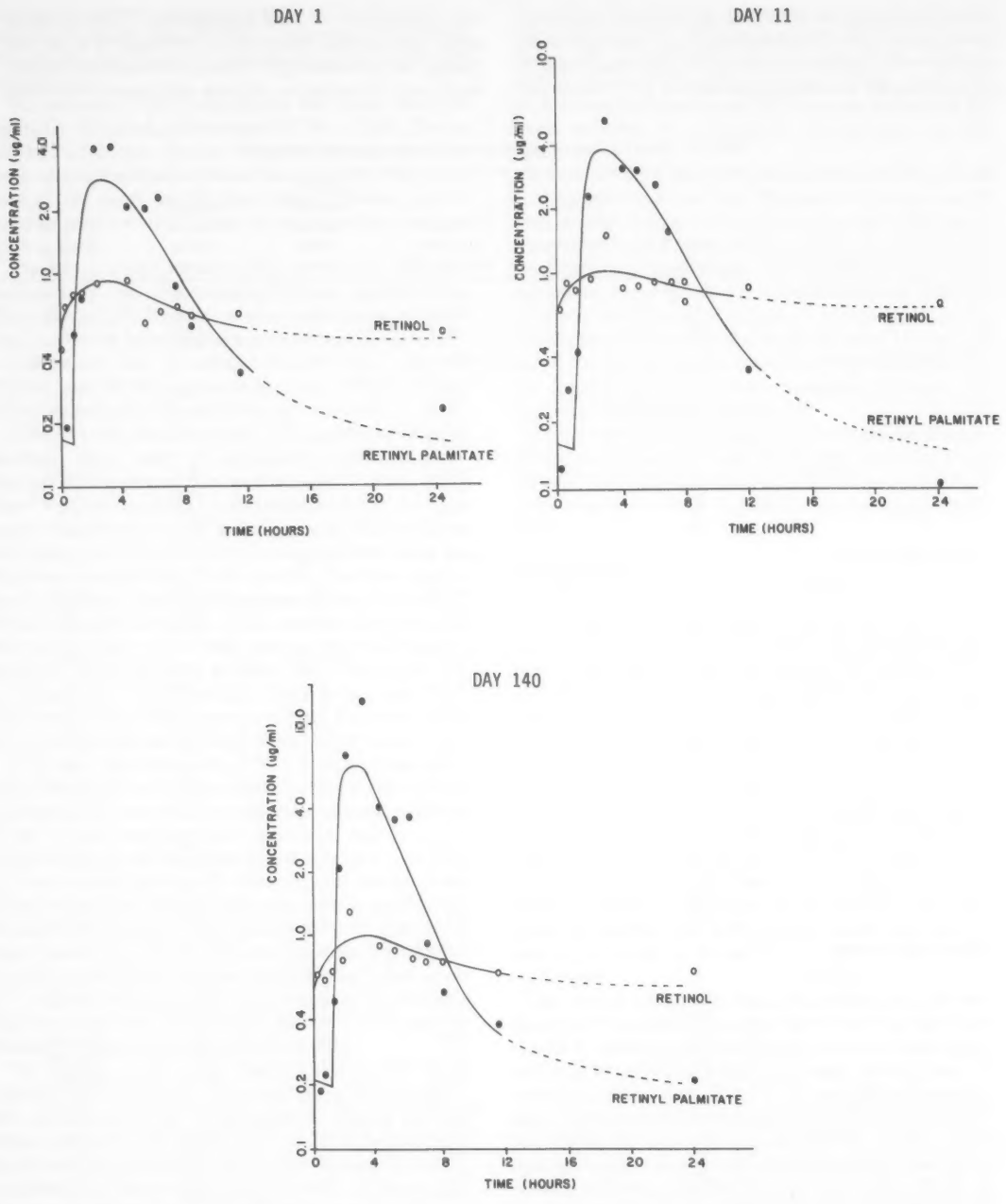

FIGURE 2.—Computer-plotted plasma concentration/time curves for R and RP in Patient 1 receiving 100,000 units/m^2/day of R. Patient was studied on Days 1, 11, and 140. Plasma R concentrations showed little change with chronic therapy, while those of RP progressively increased.

and found peak thoracic duct lymphatic concentration between 3 and 4 hours with the majority of ^{14}C occurring as the retinyl esters, RP and RS.

The progressive plasma accumulation of RP reflects its prolonged plasma $t_{½}$. Four patients were studied with prolonged blood sampling up to 120 hours after a single oral dose. Even with prolonged sampling, the terminal phase $t_{½}$ could still not be accurately determined. Both hepatic storage acting as a "deep" tissue compartment and the slow hepatic clearance of high plasma concentrations likely explain the prolonged RP elimination phase plasma $t_{½}$. Because of its prolonged $t_{½}$, low RP plasma concentrations (< 100 ng/ml) are unlikely in subjects taking chronic high doses of oral R. Hence, determination of the plasma RP concentration may be a mechanism to monitor compliance of subjects receiving oral R.

RS plasma concentrations also increased after oral R. Although concentrations could not be determined, the peak areas ranged from 20% to 40% of the simultaneous RP peak. The activity and metabolism of RS have not been investigated but are probably similar to RP. Additional HPLC peaks were seen on the chromatograms both previous to and after RS and RP. Although not identified, these peaks likely represent additional retinyl fatty acid esters.

When interpreting plasma retinoid kinetics, it is important to consider the status of the patients' retinoid stores. This is particularly important when studying cancer patients, since prospective and retrospective studies have shown that these patients may have low plasma R concentrations (21–25). The etiology of the low R concentration in this population is unknown and may relate to low retinoid intake (with resulting decreased retinoid stores) or abnormal retinoid absorption, transport, metabolism, storage, or excretion. Hence, studies with retinoids in cancer patients are likely to reflect their underlying potential deficiency state. In our study, pretreatment R concentrations (table 2, Day 1 of treatment) were normal in five patients. These patients showed no significant change in plasma R concentration during treatment.

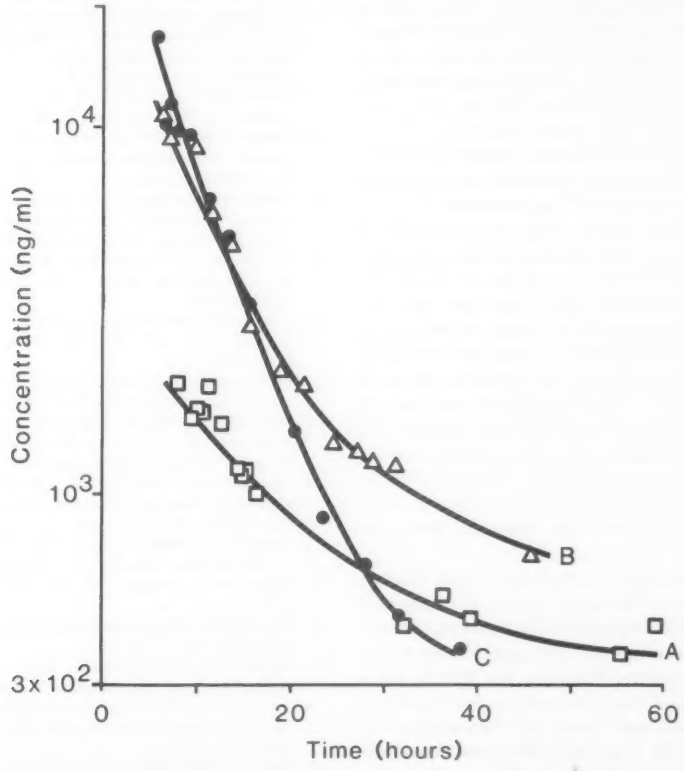

FIGURE 3.—Computer-plotted plasma concentration/time curve for RP in Patients 10–12 receiving 200,000 units/m^2 of R as a single dose at time 0. Curve A = Patient 10; Curve B = Patient 11; Curve C = Patient 12.

Three patients (Patients 4-6) had low pretreatment plasma R concentration and all had a rise in plasma R concentration. Hence, in these cancer patients, high doses of oral retinol increased low plasma R concentrations. This may simply represent correction of a poor dietary intake or may represent the effect of a pharmacologic dose of R on an underlying metabolic defect.

All patients remained on their normal diet without additional vitamin supplementation for the duration of the study. This diet was uncontrolled but contained an estimated 1000-5000 units of vitamin A, < 5% of the supplemental R intake. Although our assay could not distinguish between dietary and supplemental R, it is unlikely that the small amounts of dietary R significantly influenced the plasma concentrations of R, RP, or RS.

Despite our toxicologic and plasma concentration monitoring, the relationship between retinoid toxicity and plasma retinyl ester concentration remains unclear. Smith and Goodman (26) studied the plasma concentrations of both RBP-bound retinol and the unbound retinyl esters in three patients presenting with vitamin A toxicity. Their studies suggested that retinoid toxicity was related to the plasma concentration of the retinyl esters and proposed that toxicity was mediated via the nonspecific surface active properties of these unbound esters. However, only one of our eight patients developed acute dose-limiting retinoid toxic effects within the 1-12-month study period (14). This patient received an R dose of 150,000 units/m^2/day for 4 months (Patient 3). On Day 91 of treatment, she had a peak plasma RP concentration of 21.2 μg/ml and an average R concentration of 603.3 ng/ml. None of the three patients who received 200,000 units/m^2 developed acute toxic effects; all three had higher plasma RP concentrations but similar R concentrations. Although our data are limited, we have been unable to directly relate plasma retinyl ester concentration to acute R toxicity. It is important to note that our study was prospective as opposed to the retrospective review of Smith and Goodman (26). High concentrations of free retinyl ester may be associated with retinoid toxicity, but our data suggest that a high retinyl ester plasma concentration is not the sole cause of toxicity. These studies do not reflect on chronic retinoid toxicity, which is well-documented in patients receiving similar R doses for longer periods of time.

We have shown that high doses of oral R lead to high plasma concentrations of the retinyl esters with minimal changes in plasma R concentration. High plasma concentrations of the retinyl esters did not appear to correlate with acute clinical toxicity. However, the retinol doses used in this trial when administered for prolonged periods are clearly associated with the development of chronic retinoid toxicity. It is likely that lower doses will be required if more chronic administration is anticipated.

Since the retinyl esters, including RP, have shown significant "antiproliferative" activity, R supplementation may prove an effective chemopreventive agent by both correcting potential underlying R deficiency and providing pharmacologic plasma concentrations of RP.

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Characteristics of Inhibition of Human Renal Adenosine Triphosphatases by Cisplatin and Chloroplatinic Acid[1]

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Cisplatin and chloroplatinic acid were examined for in vitro inhibition of human renal microsomal adenosine triphosphatases activated by $Na^+ + K^+ + Mg^{2+}$, Mg^{2+}, and Ca^{2+}. The concentrations of cisplatin to inhibit 50% of activity (I50) were $\sim 7 \times 10^{-4}$ M for all enzymes studied; I50s of chloroplatinic acid were on the order of 10^{-5} M for $Na^+ + K^+ + Mg^{2+}$ ATPase and Ca^{2+} ATPase and 10^{-7} M for Mg^{2+} ATPase in the presence of $Na^+ + K^+$ + ouabain. Inhibition of $Na^+ + K^+ + Mg^{2+}$ ATPase by cisplatin or chloroplatinic acid was reversible and was not altered by varying Na^+, K^+, or Mg^{2+} concentrations; ATP or MgATP increased inhibition by cisplatin but not by chloroplatinic acid; acidic pH of 6.8 lowered inhibition by chloroplatinic acid but not by cisplatin. Cysteine, glutathione (–SH reagents), and ascorbic acid greatly reduced inhibition of all enzymes studied by chloroplatinic acid; in the case of cisplatin, –SH reagents had only a minimal protective effect but ascorbic acid somewhat increased inhibition. Methionine greatly increased inhibition by cisplatin but provided minimal protection in the case of chloroplatinic acid. In view of the hypothesis that inhibition of renal $Na^+ + K^+$ ATPase may be associated with tubular damage, the inhibition of $Na^+ + K^+$ ATPase may be relevant to the mechanism of platinum toxicity. [Cancer Treat Rep 68:1135–1141, 1984]

Cisplatin, a cancer chemotherapeutic drug containing platinum, has toxic effects on several tissues including cardiac, hematopoietic, neural, auditory, gastrointestinal, and renal (1-3). Following administration of cisplatin, platinum concentrates in the renal cortex and renal insufficiency may result (3-10). Cisplatin or its metabolite (if any) is actively secreted by the kidney, possibly by the organic base mechanism (10). The general mechanism of cellular toxicity appears to be the binding of the platinum to -SH groups in the proximal tubule with possible inhibition of enzymes and alterations of membrane permeability (9-11). The incidence of renal damage may be reduced by maneuvers which prevent excessive concentration of platinum in the kidney, ie, lengthening the time of cisplatin infusion, use of hydration, and diuresis (10). Heavy metal chelators do not appear to protect (5,11). Other methods of reducing cisplatin nephrotoxicity are under investigation (12), since protection of the kidney would increase the therapeutic index of the drug.

Platinum-containing compounds inhibit Ca^{2+} ATPase of sarcoplasmic reticulum (13-15) and renal $Na^+ + K^+$ ATPase in experimental animals (16,17). $Na^+ + K^+$ ATPase, and possibly other ATPases, function in electrolyte homeostasis (18) and renal damage by cisplatin (17), and other metals such as mercury, lead, cadmium, uranium, gold, and vanadium may be associated with inhibition of $Na^+ + K^+$ ATPase (19,20). Guarino et al (16) suggested that other major sites of toxicity (inner ear, nerves, and gastrointestinal tract) may also involve inhibition of ATPase by cisplatin.

The purpose of this study was to characterize the effects of cisplatin and chloroplatinic acid on human kidney ATPases and to examine the possibility of changing the inhibition of these enzymes by certain agents of physiological significance. Chloroplatinic acid was used in this study for comparison with cisplatin because of its greater nephrotoxicity (21) and different oxidation state of platinum (+4 vs +2, respectively, the principal oxidation states of platinum) (22). Chloroplatinic acid is not used as a drug but could be of toxicological interest. An abstract of this work has been published (23).

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METHODS

Human kidneys, in excellent condition, but rejected for transplant for technical reasons, were used in this study. Microsomal preparations were made as described previously (24). Briefly, whole kidney tissue homogenates were prepared 1:10 in a sucrose solution containing tris (hydroxymethyl)-aminomethane, disodium edetate, and sodium deoxycholate. These homogenates were used for obtaining microsomal fractions by differential centrifugation. The enzyme activity was measured by the rate of release of phosphate from exogenous ATP at 37°C. The incubation mixture contained diluted microsomal fragments, 3mM ATP, cations (as described in the Results section), and 40 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer at pH 7.4. Varying the concentration of HEPES buffer between 20 and 80 mM did not alter the inhibition by the compounds studied. Enzyme activity inhibited by 1×10^{-4} M ouabain represented $Na^+ + K^+$ ATPase activity. The properties of human renal ATPase were described previously (25).

Cisplatin was obtained from Bristol Laboratories, Syracuse, NY. Reagent grade chloroplatinic acid (H_2PtCl_6) was obtained from Fisher Scientific Co, Houston, TX. Aqueous solutions of these compounds were preincubated at 37°C with the enzyme and electrolytes, without ATP, for 30 minutes to give maximal enzyme inhibition for each concentration of platinum. The addition of ATP started the reaction.

Results are expressed as mean ± SD where applicable. Groups were compared by two-sample, unpaired, t test. P value of 0.05 was accepted as the limit of significance. I50 values were determined using the allfit program of DeLean et al (26).

RESULTS

Figure 1 shows that ATPases studied which are defined in the legend were more sensitive to inhibition by chloroplatinic acid than by cisplatin. Fifty percent of total (mainly $Na^+ + K^+$) ATPase activity was inhibited by 8×10^{-4} M (I50) cisplatin or 1×10^{-5} M chloroplatinic acid. Total (mainly $Na^+ + K^+$) ATPase activity was slightly, but significantly ($P < 0.01$), less sensitive to cisplatin than were the other ATPase activities.

Ouabain-insensitive ATPase activity was 40 times more sensitive to inhibition by chloroplatinic acid (I50 4×10^{-7} M) than the total (mainly $Na^+ + K^+$) ATPase activity. Mg^{2+} ATPase was 40 times less sensitive to chloroplatinic acid (I50 1×10^{-5} M) than ouabain-insensitive ATPase. However, when Na^+ (100 mM) and K^+ (20 mM) were added to the incubation medium of the Mg^{2+} ATPase preparation, the sensitivity of the enzyme to chloroplatinic acid increased to that of ouabain-insensitive ATPase preparation which contained Na^+ and K^+ (fig 1B; table 1). Na^+ and K^+ in the ATPase preparation in figure 1B did not increase the sensitivity of ouabain-insensitive ATPase to cisplatin over that of Mg^{2+} ATPase.

The I50 of chloroplatinic acid (5×10^{-5} M) was fivefold higher for Ca^{2+} ATPase than the other ATPase studied. In contrast to Mg^{2+} ATPase, the sensitivity of Ca^{2+} ATPase was not increased by addition of Na^+ and K^+ (data not shown).

Components of the enzyme reaction modify the response of $Na^+ + K^+$ ATPase to some other metals (27-29), and were examined for their effects on platinum compounds inhibition. Varying concentrations of Na^+ (20-150 mM), K^+ (2-20 mM), and Mg^{2+} (3-9 mM) did not alter inhibition of total enzyme activity by a constant concentration of either cisplatin or chloroplatinic acid (data not shown).

In two experiments, increasing concentrations of MgATP (2-6 mM) or ATP (1-6 mM) increased inhibition of total ATPase activity by 5×10^{-4} M cisplatin from 20% to 70%, but did not alter the inhibition by chloroplatinic acid (data not shown).

$Na^+ + K^+$ ATPase inhibition by other metals varies with the pH of the medium (29,30). In the present study, inhibition by a constant concentration of cisplatin did not vary over a pH range of 6.8-8.0. In two experiments, we observed inhibition by 1×10^{-5} M chloroplatinic acid to decrease with acidity (56% inhibition at pH 8.0 vs 35% inhibition at pH 6.8) (data not shown).

Ackermann and Potter (31) plots show that fractional inhibition of total (mainly $Na^+ + K^+$-dependent) and ouabain-insensitive ATPases by constant concentrations of

TABLE 1.—Effect of Na^+ and K^+ on the inhibition of Mg^{2+} ATPase by chloroplatinic acid *

	Chloroplatinic acid (M)		
	10^{-7}	10^{-6}	10^{-5}
$MgCl_2$	30 ± 4	44 ± 5	55 ± 3
$MgCl_2$ + NaCl	45 ± 2	63 ± 3	71 ± 4
$MgCl_2$ + KCl	40 ± 3	57 ± 5	65 ± 5
$MgCl_2$ + NaCl + KCl	49 ± 4	66 ± 5	73 ± 6

* Values in table = % enzyme inhibition. Concentrations of the ions were 3 mM $MgCl_2$, 100 mM NaCl, and 20 mM KCl. Ouabain (10^{-4} M) was present in all tubes to inhibit residual $Na^+ + K^+$ ATPase activity. N = 5.

either platinum compound was independent of enzyme concentration (fig 2). This type of inhibition indicates a reversible nature of enzyme-inhibitor binding.

Since platinum has a high affinity for sulfhydryl ligands (9) and complexes of platinum bind sulfur-containing amino acids during interactions with proteins (13), several compounds containing sulfur (cysteine, glutathione, and methionine) were included in our preparation. Figure 3 shows that cysteine (10^{-5} to 10^{-3} M) greatly decreased inhibition of all ATPase activities studied by chloroplatinic acid in a concentration-dependent manner. A concentration of cysteine as high as 10^{-3} M had only minimal protective effect on inhibition by cisplatin. Glutathione (10^{-5} to 10^{-3} M) produced effects similar to those of cysteine (data not shown). Methionine (10^{-4} to 10^{-2} M) greatly increased inhibition by cisplatin of the ATPases in a concentration-dependent manner, but slightly decreased inhibition by chloroplatinic acid (fig 4).

The reducing agent, ascorbic acid, has been shown to alter inhibition of ATPase by other metals (29,30). In the present study, ascorbic acid slightly increased the inhibi-

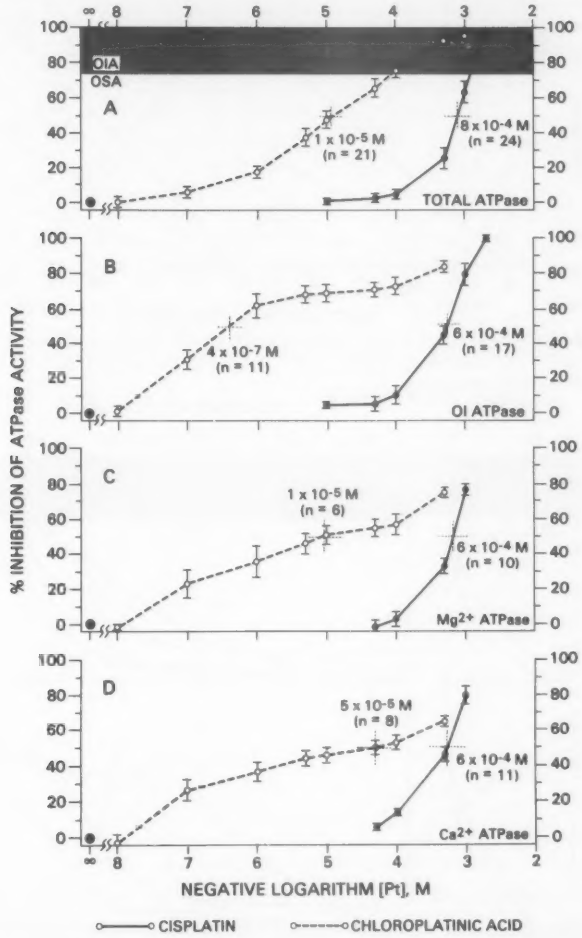

FIGURE 1.—Effect of cisplatin and chloroplatinic acid on activity of ATPases under various ionic conditions. Cross lines show molar concentrations of platinum causing 50% inhibition (I50) of enzyme activity. **A.** Total ATPase activity in presence of 100 mM NaCl, 20 mM KCl, and 3 mM $MgCl_2$. Shaded and clear areas separate ouabain-sensitive (OSA) from ouabain-insensitive ATPase activity (OIA). Note that total ATPase activity represents mainly (73%) $Na^+ + K^+$ ATPase activity. **B.** OIA under ionic conditions as in A with $Na^+ + K^+$ ATPase activity inhibited by 10^{-4} M ouabain. **C.** Mg^{2+} ATPase activity in presence of 3 mM $MgCl_2$ and 10^{-4} M ouabain to inhibit any residual $Na^+ + K^+$ ATPase activity. **D.** Ca^{2+} ATPase activity in presence of 3 mM $CaCl_2$ and 10^{-4} M ouabain to inhibit any residual $Na^+ + K^+$ ATPase activity. Average protein content of enzyme preparations was 26 μg/ml in A, B, and C, and 46 μg/ml in D.

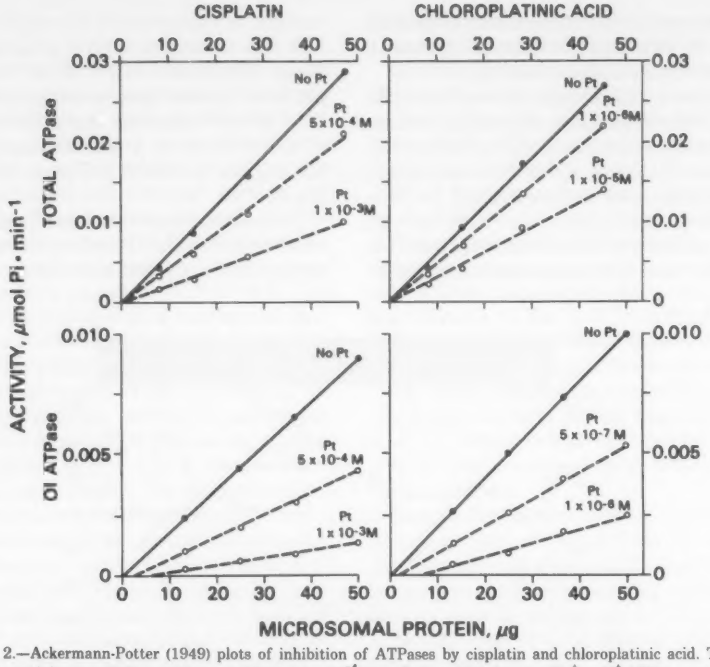

FIGURE 2.—Ackermann-Potter (1949) plots of inhibition of ATPases by cisplatin and chloroplatinic acid. Total ATPase activity was 74% sensitive to inhibition by 10^{-4} M ouabain, which denotes $Na^+ + K^+$ ATPase activity. Each graph represents 3-5 experiments.

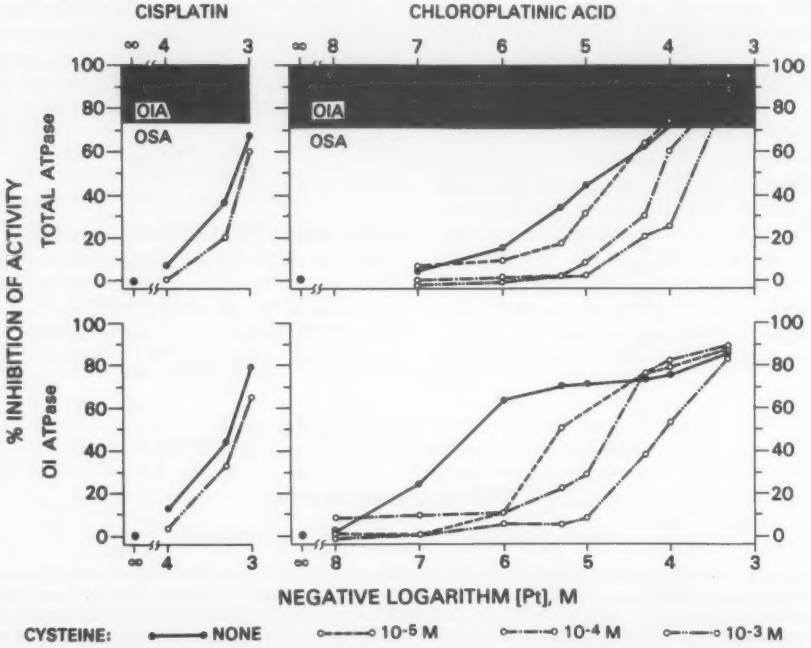

FIGURE 3.—Effects of cysteine on inhibition of ATPases by cisplatin and chloroplatinic acid. Enzymes are defined in legend to fig 1. Cysteine was preincubated with platinum compounds in a mixture of buffer and electrolytes at 37°C for 30 mins before ATP was added to start the enzyme reaction. Results with Mg^{2+} or Ca^{2+} ATPase were similar to those with OI ATPase (data not shown). Each graph represents 2-3 experiments.

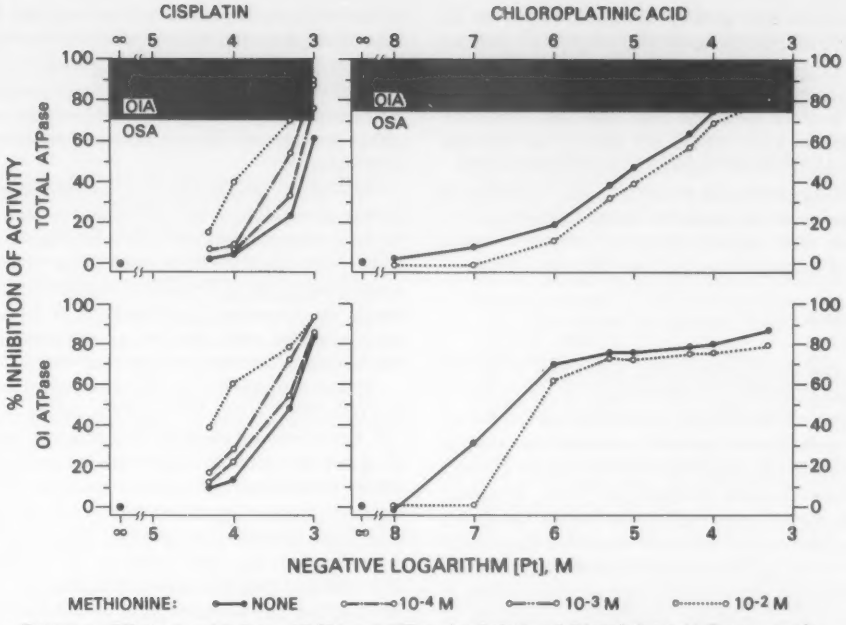

FIGURE 4.—Effects of methionine on inhibition of ATPases by cisplatin and chloroplatinic acid. Enzymes are defined in legends to fig 1, and the method of incubation is described in fig 3. Each graph represents 2-3 experiments.

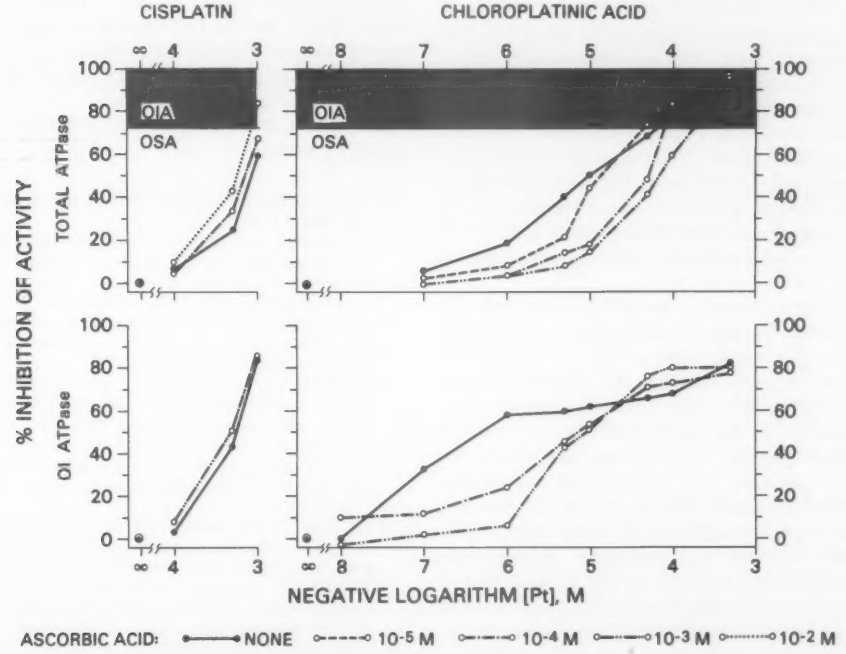

FIGURE 5.—Effects of ascorbic acid on inhibition of ATPases by cisplatin and chloroplatinic acid. Enzymes are defined in legends to fig 1, and the method of incubation is described in fig 3. Each graph represents 2-3 experiments.

tion by cisplatin but greatly decreased inhibition by chloroplatinic acid in a concentration-dependent manner (fig 5).

Platinum complexes with the imidazole ring of histidine (22). However, we found that even high concentrations of histidine (10^{-2} M) did not protect the ATPases from inhibition by either cisplatin or chloroplatinic acid.

The following agents did not modify the inhibition of the ATPases by either platinum compound: bovine serum albumin (200 μg/ml), edetate (10^{-3} M), thiamine (10^{-2} M), and sodium sulfate (10^{-2} M) (data not shown).

DISCUSSION

Cisplatin and chloroplatinic acid were found to inhibit in vitro renal ATPases activated by $Na^+ + K^+ + Mg^{2+}$, Mg^{2+}, and Ca^{2+}. The ability of platinum to inhibit $Na^+ + K^+$ ATPase activity is of interest in view of the evidence that many agents and conditions known to cause Fanconi syndrome interfere with the renal $Na^+ + K^+$ transport system (19). Since cardiac glycosides do not commonly produce renal damage, it has been suggested that inhibition of $Na^+ + K^+$ ATPase cannot be involved in the pathology. However, digitalization (or sublethal doses of cardiac glycosides) produces insufficient inhibition of $Na^+ + K^+$ ATPase to cause direct alteration of kidney function (18,32). The fact that platinum accumulates in the kidneys of animals treated with cisplatin (4-7) suggests that under certain circumstances these enzymes may be inhibited in vivo. Little is known about biotransformation of cisplatin (10), and the product could have different potency than the parent compound. Also, chronic administration of cisplatin may have a cumulative effect on the enzyme if the experience with vanadium is applicable. Unlike acute exposure, feeding of vanadate to chickens for several weeks reduced $Na^+ + K^+$ ATPase activity in renal homogenates and caused tubular damage with increasing concentration of dietary vanadate (20,33).

Cisplatin causes polyuria in experimental animals (16) which may be due to reduced ADH output, to inhibition of tubular Na^+Cl^- reabsorption and $Na^+ + K^+$ ATPase activity, or to less specific damage. McCarley and Dennis (11) argue that cisplatin may primarily change membrane permeability to Na^+ in renal tubules. It is possible that renal Mg^{2+} and Ca^{2+} ATPases function in reabsorption of Mg^{2+} and Ca^{2+} and the observed hypomagnesemia and hypocalcemia in patients treated with cisplatin (1,10,34) may be due to inhibition of these enzymes. The hypocalcemia reported in experimental chloroplatinic acid nephropathy (21) may also be due to inhibition of Ca^{2+} ATPase. Alternatively, the urinary loss of Ca^{2+} and Mg^{2+} may be linked to inhibition of tubular Na^+ transport.

Platinum reacts with compounds containing sulfur (22). In our study, sulfhydryl reagents cysteine and glutathione only slightly protected the enzymes from inhibition by cisplatin, but were very effective in decreasing inhibition by chloroplatinic acid. Therefore, dimercaprol (-SH chelating agent) may prove useful as an antidote in poisoning by chloroplatinic acid. Consistent with our results, dimercaprol did not prevent cisplatin nephrotoxicity in rats (5).

Methionine, which contains a methylated sulfhydryl group, greatly increased inhibition by cisplatin, but not that by chloroplatinic acid. This finding raises the possibility that nephrotoxicity induced by cisplatin may be augmented by dietary intake of methionine. Therefore, it would be interesting to determine if patients treated with cisplatin who consume a vegetarian diet, low in methionine (35), show less nephrotoxicity.

Platinic chloride [$PtCl_4^{-2}$(II)] was less potent in inhibiting renal $Na^+ + K^+$ ATPase from guinea pigs (17) than the same concentration of chloroplatinic acid [H_2PtCl_6 (IV)] but was similar in potency to cisplatin (II) in our study. Since ascorbic acid is a reducing agent and decreased inhibition by chloroplatinic acid, we propose that ascorbate reduced chloroplatinic acid according to the following formula: $PtCl_6^{2-}$ (IV) + $2e^- \rightleftharpoons PtCl_4^{2-}$ (II) + $2Cl^-$ (36) and that the degree of inhibition of the $Na^+ + K^+$ ATPase may be determined by the oxidation state of platinum. Therefore, ascorbic acid may prove useful to treat poisoning with Pt^{4+} compounds on the theory that Pt^{4+} would be reduced to a less toxic +2 state. On the other hand, our finding that high concentrations of ascorbate increased ATPase inhibition by cisplatin raises the possibility that an excessive intake of the vitamin could augment cisplatin toxicity.

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Effect of the Interval Between Exposures to Cytarabine on Its Cytotoxic Action on HL-60 Myeloid Leukemic Cells[1,2]

Jean-Marie Leclerc and Richard L. Momparler[3,4,*]

As an in vitro model for the chemotherapy of acute myeloid leukemia with cytarabine (ARA-C), the cytotoxicity of this drug was investigated using a human promyelocytic cell line (HL-60) and different drug schedules. The continuous exposure to ARA-C was shown to be more cytotoxic than the intermittent exposures at identical concentrations or under conditions where the concentrations multiplied by time of exposure were the same. In a comparison of exposures, as the intervals between drug exposures were reduced, the cytotoxicity of ARA-C increased. Flow microfluorometric analysis of DNA content showed that a 3-hour exposure to ARA-C every 6 hours produced a greater accumulation of cells in S phase than the same exposure repeated every 12 hours. After a 3-hour exposure to 20 μg/ml of ARA-C, the cells recovered 38% and 85% of their capacity to synthesize DNA within 4 and 8 hours, respectively. These findings can be explained in part by the short half-life of the intracellular nucleotide pool of ARA-CTP in these cells (about 60 minutes). These data indicate that when there is a prolongation of the interval between exposures to ARA-C, a greater fraction of cells recover from the inhibitory effects of this drug and escape its cytotoxic action. These observations may be important with respect to the design of more optimal schedules of high-dose ARA-C therapy for the treatment of patients with acute leukemia. [Cancer Treat Rep 68:1143–1148, 1984]

Cytarabine (ARA-C) is an important drug for the treatment of acute leukemia (1). The conventional dose schedule for ARA-C is 100–200 mg/m^2/day administered as a continuous iv infusion for several days (1). Drug resistance develops frequently with this type of therapy, presumably due to the presence of very low plasma concentrations of ARA-C (2). To overcome this problem, the rationale for using high-dose ARA-C therapy was proposed (2).

Patients with acute leukemia who are resistant to conventional chemotherapy have been treated with high-dose ARA-C with some success. The dose schedule used was 3 g/m^2 (1–3-hour iv infusion) administered every 12 hours for four to 12 cycles (3–6). Although these results with high-dose ARA-C are very promising, future work should be directed toward the optimization of the schedule of administration.

Studies on L1210 leukemic cells have shown that the antineoplastic activity of ARA-C is very schedule-dependent, especially with respect to the interval between drug treatment (7–9). In this report, we have studied the in vitro cytotoxic action of different schedules of ARA-C on the human myeloid leukemic cell line HL-60. We have found that the length of the interval between ARA-C exposures greatly influences its cytotoxic action. We have also made an attempt to understand the mechanism responsible for this effect.

MATERIALS AND METHODS

Chemicals.—Nonradioactive nucleotides were obtained from P. L. Biochemicals (Milwaukee, WI). [Methyl-^{3}H]thymidine (20 Ci/mmol) and [^{3}H]ARA-C (15.5 Ci/mmol) were purchased from NEN Canada (Montreal). ARA-C was obtained from The Upjohn Company (Kalamazoo, MI) and tetrahydrouridine (THU) was obtained from Calbiochem (La Jolla, CA).

Cell culture.—HL-60 human promyelocytic leukemic

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cells (10) were obtained from Dr. R. C. Gallo, National Institutes of Health, Bethesda, MD. The cells were grown as a suspension culture in minimal essential medium (MEM) supplemented with nonessential amino acids (Grand Island Biological Co, New York) and 15% heat-inactivated fetal calf serum (Flow Labs, Mississauga, Ontario, Canada). Twice weekly the cells were diluted to 200,000 cells/ml in fresh medium. The cells had a doubling time of 35–40 hours. Cell survival after drug exposure was measured by cloning in 0.8% methylcellulose containing MEM plus 20% heat-inactivated fetal calf serum. After each drug exposure, treated and untreated cells were washed twice with MEM medium. After 14 days of incubation at 37°C in a fully humidified atmosphere containing 5% CO_2 in air, the number of colonies (> 50 cells) were counted using an inverted microscope at 50× magnification. Viability was obtained by dividing the number of colonies obtained with the treated cells by those produced with untreated cells. Cloning efficiency of untreated HL-60 cells ranged from 25% to 35% and was not affected by repeated washings with MEM medium.

[^{3}H]thymidine incorporation into DNA.—HL-60 cells (2×10^5/ml) were placed in 2 ml of MEM containing 5% dialyzed fetal calf serum, 1.0 μCi of [methyl-^{3}H]thymidine (20 Ci/mmol), and the indicated concentrations ARA-C or THU. Tubes were incubated in a shaker bath at 37°C for 2 hours and the amount of radioactivity incorporated into DNA was determined as described previously (11). The flash [^{3}H]thymidine labeling index for HL-60 cells was reported to be 52% (12).

Intracellular nucleotide pools of ARA-C.—HL-60 cells (8×10^6 cells in 4 ml of MEM plus 5% dialyzed fetal calf serum) were incubated with 4 μCi of [^{3}H]ARA-C (10^{-6} M) for 3 hours at 37°C. The cells were then washed twice, resuspended in fresh MEM medium, and incubated at 37°C. At different times, the cells were centrifuged at 1000 rpm for 3 minutes, the supernatant was discarded, and the cell pellet was resuspended in 1.5 ml of cold 0.9% NaCl. After centrifugation at 12,000 g for 3 seconds, the cell pellet was submitted to mechanical breakage in 60% ethanol. The ethanol supernatant was obtained after centrifugation at 12,000 g for 15 minutes. The determination of [^{3}H]ARA-C metabolites was performed by thin-layer chromatography using DEAE cellulose plates (UNIPLATE, 250 μm, Analtech). Cold carrier (20 μl) containing cytidine, CMP, CDP, CTP, and UMP at concentrations of 10 mM each was spotted at the origin with 10 or 15 μl of ethanol extract, and development was performed in potassium acetate, 0.2 M, pH 4.5. After the development, plates were dried, nucleotides were detected by UV, cut out, and diluted in 1 ml of 0.1 N HCl and 10 ml of Aquasol for radioactivity determination.

Flow microfluorometry.—Fixation and staining of cells for flow microfluorometry were done according to methods reported previously by Barlogie et al (13). In brief, 2 ml of washed cells at 5×10^3/ml were centrifuged at 1200 rpm for 5 minutes and the cell pellet was resuspended in 70% ethanol. After fixation, the cells were resuspended in 1 ml of ethidium bromide-mithramycin dye solution. Flow microfluorometry was performed on a Becton-Dickinson FACS III flow cytometer. DNA distribution histograms were recorded as an expanded X-Y scale by a computer.

RESULTS

The continuous treatment of HL-60 leukemic cells with ARA-C at 0.2, 2.0, and 20 μg/ml for 48 hours produced 1-, 2-, and 3-log cell kill, respectively (fig 1A). The intermittent exposures to ARA-C (15 μg/ml, 3 hours) were less cytotoxic as the intervals between treatments were prolonged (fig 1B). The experimental points indicate the end of exposure time. Treatment of the cells with ARA-C at 4.6 μg/ml continuously for 39 hours killed 1.0 log more leukemic cells than intermittent exposures of 3 hours at intervals of 6 hours at a concentration of 8.5 μg/ml for 39 hours under conditions where the concentrations multiplied by time of exposure (C × T) for both treatments were identical (fig 1C). When the interval between the 3-hour exposures was 12 hours with ARA-C at 15 μg/ml for 60 hours, a continuous exposure of ARA-C at 1.5 μg/ml for 60 hours produced about 3 log greater cell kill (data not shown).

Typical DNA histograms of untreated HL-60 cells (0 hours) are shown in figures 2A and 2B. The first peak corresponds to cells with a 2n content of DNA and represents cells in G_1 phase. The cells were exposed for 3 hours to high doses of ARA-C (15 μg/ml) at intervals of 6 hours (fig 2A) or 12 hours (fig 2B). DNA histograms obtained just before the next treatment showed changes in population distributions. Just before the second treatment, cells exposed to ARA-C at 6-hour intervals were distributed principally in S phase (fig 2A; 6 hours). Cells exposed at 12-hour intervals (fig 2B; 12 hours) showed a large accumulation in G_1 phase. Before the third treatment, cells treated every 6 hours were primarily in S phase compared to cells treated every 12 hours which did not accumulate in any particular phase. Before the fourth treatment, we did not observe differences in DNA distributions between both schedules. DNA histograms of cells continuously exposed to ARA-C were very similar to those treated every 6 hours (data not shown).

Treatment of HL-60 leukemic cells for 3 hours with a high dose of ARA-C (15 μg/ml) produced an 89% inhibition of DNA synthesis 1 hour after the removal of the drug (table 1). Eight hours after exposure to ARA-C, the treated cells were almost as active as the untreated cells in their capacity to incorporate [^{3}H]thymidine into DNA. The recovery from inhibition of DNA synthesis was practically linear and was not significantly modified by the presence of THU (10^{-6} M) during and after the ARA-C treatment.

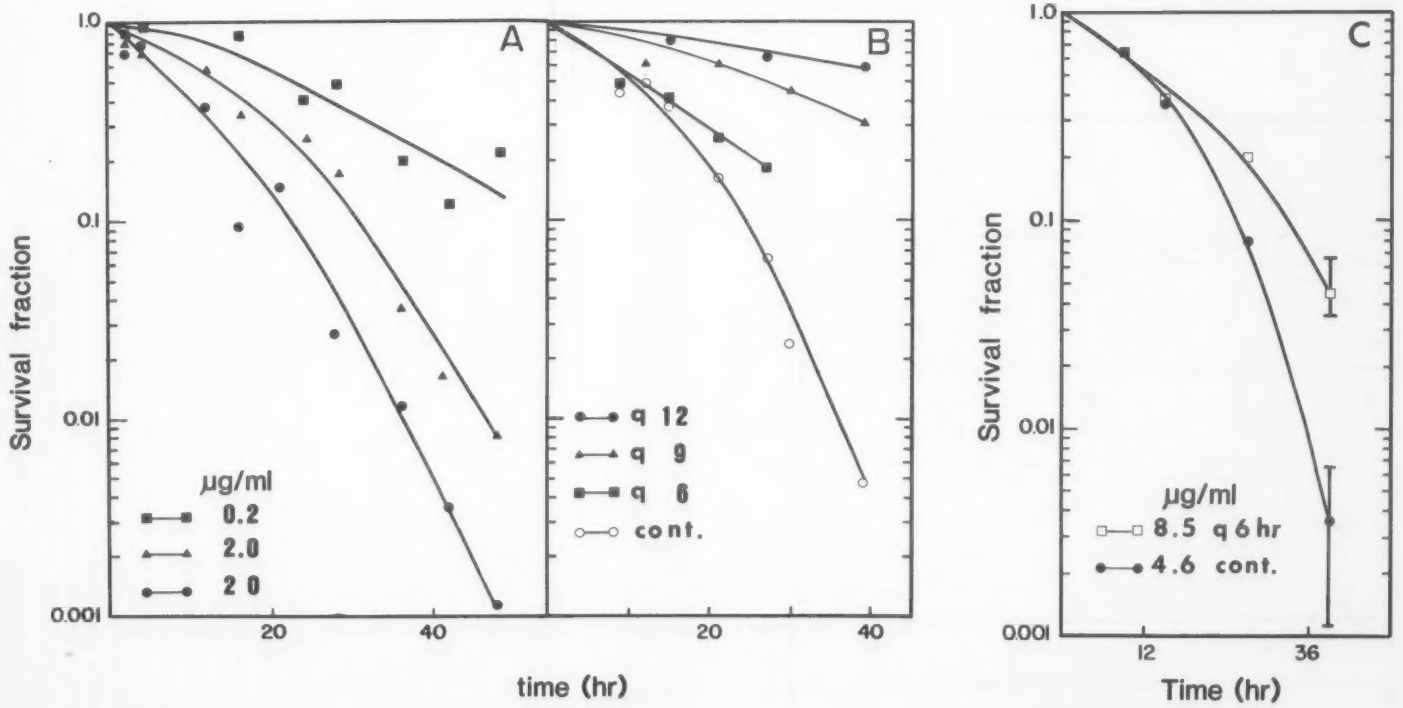

FIGURE 1.—Cytotoxicity of different schedules of ARA-C on HL-60 leukemic cells. A: Cells were exposed continuously to 0.2 (■), 2.0 (▲), or 20 μg/ml (●) of ARA-C for the time indicated. B: Cells were exposed (○) continuously or for 3 hrs intermittently to 15 μg/ml of ARA-C every 6 hrs for 4 treatments (■), every 9 hrs for 4 treatments (▲), or every 12 hours for 3 treatments (●). C: Comparison of the cytotoxicity of a continuous or intermittent exposure to ARA-C under conditions where the C × T was identical. ● = 4.6 μg/ml continuously for 39 hrs; □ = 8.5 -μg/ml 3-hr exposure intermittently every 6 hrs × 7 for 39 hrs. Symbol with vertical bar represents mean ± SD.

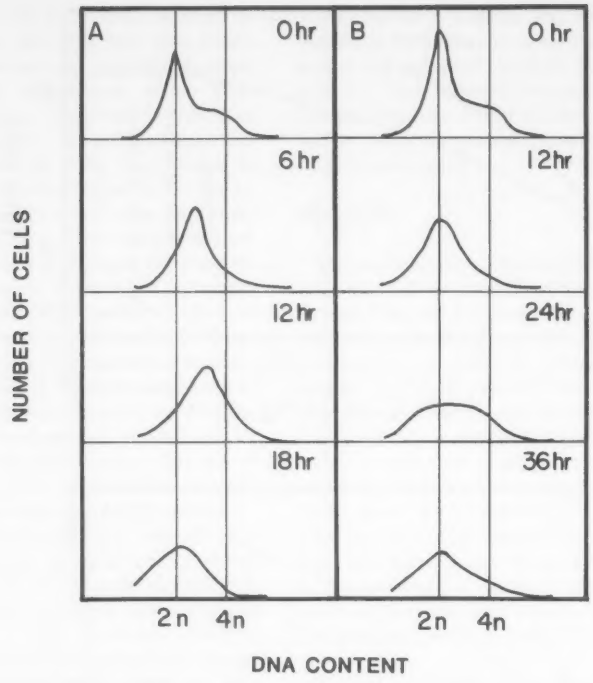

FIGURE 2.—Effect of different schedules of ARA-C exposure on DNA histogram of HL-60 leukemic cells. Cells were exposed to 15 μg/ml of ARA-C for 3 hrs every 6 hrs (A) or 12 hrs (B). At the times indicated (before drug exposure), aliquots of the cells were fixed and stained for DNA content analysis by flow microfluorometry.

HL-60 leukemic cells incubated for 3 hours with [^{3}H]ARA-C at 10^{-6} M were shown to contain 2.6 ± 0.4 nmols/10^9cells of ARA-CTP. Pools of ARA-CDP and ARA-UMP were approximately one-half that of ARA-CTP, whereas ARA-CMP was one-tenth that of ARA-CTP (fig 3). Sixty minutes after removal of [^{3}H]ARA-C from medium, the nucleotide pools were diminished by one-half but the ratios between them remained the same. The half-lives of the different nucleotides of ARA-C were all about 60 minutes.

DISCUSSION

High-dose ARA-C has been shown to be an effective therapy in patients with acute leukemia resistant to conventional therapy (3-6). The most common dose schedule

TABLE 1.—Recovery of DNA synthesis of HL-60 leukemic cells after exposure to ARA-C*

Time after ARA-C exposure (hrs)	Relative DNA synthesis (% control)†	
	ARA-C	ARA-C + THU
1	11 ± 0.3	13 ± 0.4
4	38 ± 1.6	42 ± 1.8
8	85 ± 5	91 ± 4

* The cells, 2 × 10^5/ml (2 ml), were exposed to 20 μg/ml of ARA-C ± 1 × 10^{-5} M THU for 3 hrs. After drug exposure at the times indicated, the cells were incubated with 1 μCi of [^{3}H]-thymidine (20 Ci/mmol) for 2 hrs and the radioactivity incorporated into DNA was determined. The untreated cells (control) incorporated 33,950 ± 600 cpm [^{3}H]-thymidine into DNA.

†Values = mean ± SD.

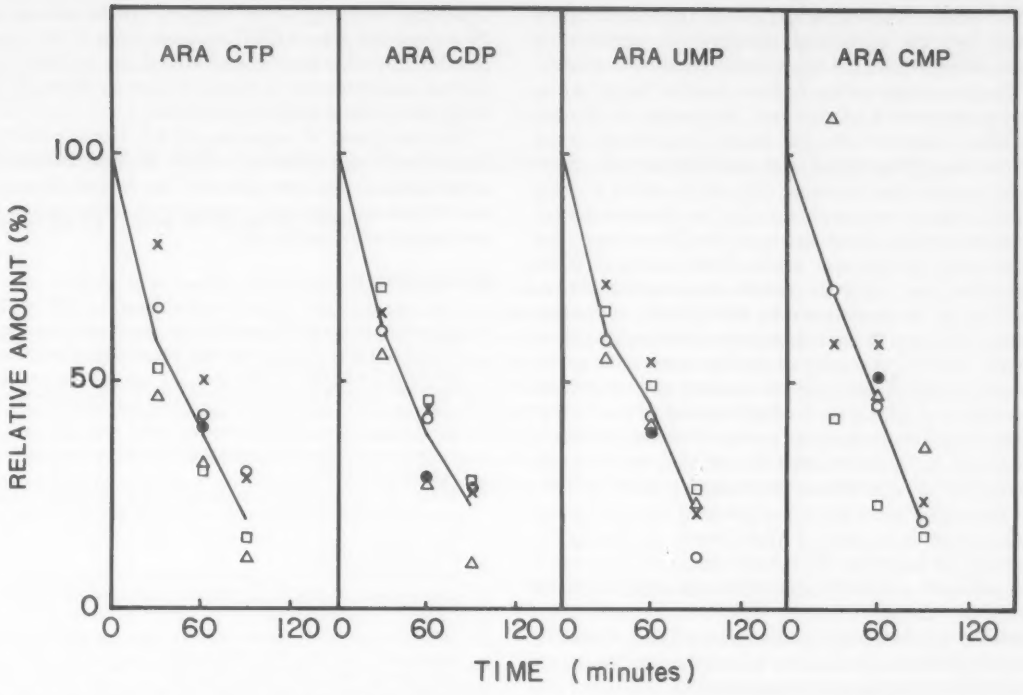

FIGURE 3.—Metabolism of ARA-C nucleotides in HL-60 leukemic cells. The cells, 2×10^6/ml (4 ml), were incubated with 4 μCi of [^{3}H]ARA-C (1×10^{-6} M) for 3 hrs. After removal of [^{3}H]ARA-C from the medium, the ARA-C nucleotides were separated by thin-layer chromatography at the times indicated. At time 0 mins, the amount of nucleotides (nmols/10^9 cells) was as follows: 2.6 ± 0.4 ARA-CTP, 1.4 ± 0.3 ARA-CDP, 0.3 ± 0.05 ARA-CMP, and 1.6 ± 0.3 ARA-UMP.

used for the high-dose therapy is 3 g/m^2 (1-3-hour iv infusion) administered every 12 hours for four to 12 cycles. Plasma concentrations of ARA-C during the high-dose infusion are in the range of 10-20 μg/ml (5), which is about 100-400-fold greater than the plasma level obtained with conventional low-dose ARA-C therapy (100-200 mg/m^2/day continuous infusion). Since ARA-C is such a schedule-dependent drug (7), future investigations should be directed toward optimizing the schedule of the high-dose therapy to improve its effectiveness.

In a mouse model with L1210 leukemia, the optimal interval between doses of ARA-C is 6-8 hours (7,8). Similar results have been obtained with L1210 leukemia cells in vitro (9). Since L1210 leukemic cells have a cell cycle of only 12 hours, it is difficult to translate these results to man, where the leukemic cell cycle is much longer (7). An important observation in these studies is that if the interval between ARA-C doses is too long, a significant fraction of the L1210 leukemic cells escape the cytotoxic action of this drug. Since there is a long interval between doses of ARA-C in the high-dose regimen (3-6), we have investigated this parameter using the human myeloid leukemic cell line HL-60 which has cell cycle characteristics (12) more similar to the human disease than the L1210 leukemia.

Comparison of the antineoplastic action of different concentrations of ARA-C for a continuous exposure showed that 20 μg/ml was significantly more cytotoxic at all exposure times than either 0.2 or 2 μg/ml for the HL-60 leukemic cells (fig 1A). The concentration of 20 μg/ml is in the range of the plasma level of ARA-C obtained during the infusion of a high dose of this drug and clearly shows the increased effectiveness of this type of therapy.

The effect of different schedules of exposure showed that a continuous exposure to 15 μg/ml of ARA-C is significantly more cytotoxic to the HL-60 leukemic cells than 3-hour exposures administered every 6, 9, or 12 hours (fig 1B). The shorter the interval between drug exposure, the greater the cytotoxic effectiveness of ARA-C. However, in this latter experiment, the C × T parameter is different for each type of treatment, making comparisons difficult. To overcome this problem, we compared the cytotoxic action of a continuous exposure with that of an intermittent exposure under conditions where the C ×

T for ARA-C was constant (fig 1C). These data clearly show that the continuous exposure was significantly more effective than the intermittent exposure to ARA-C.

The above observations indicate that the longer the interval between ARA-C exposure, the greater the fraction of HL-60 leukemic cells that escape the cytotoxic action of this drug. What is the mechanism behind this effect? It is possible that leukemic cells with a short S phase could progress completely through the phase of the cell cycle during the interval between ARA-C treatments and thus escape the cytotoxic action of this analog (2,7). On the other hand, since the lethal action of ARA-C is related to its incorporation into DNA (14,15), if the leukemic cells have the time to repair sublethal damage between ARA-C exposures, they may survive. In an attempt to understand these phenomena, we have studied the effects of ARA-C on the DNA content of the cells by flow microfluorometry and the rate of recovery from the inhibition of DNA synthesis. In addition, we have estimated the half-life of the intracellular pool of ARA-CTP.

Comparison of the DNA histograms of HL-60 leukemic cells exposed to 15 μg/ml of ARA-C every 6 hours (fig 2A) or every 12 hours (fig 2B) showed that the 6-hour schedule produced a greater accumulation of cells in S phase than the 12-hour schedule. This 6-hour intermittent schedule was too short to permit the cells to completely progress through the S phase before the next treatment and resulted in greater cytotoxicity, as mentioned above. There was a greater accumulation of cells in the G_1 phase for the 12-hour schedule, suggesting that cells in S phase recovered from the ARA-C exposure and continued to progress into the G_1 phase, a phase of the cell cycle where cells can escape the cytotoxic action of this S-phase-specific agent (16). Other investigators using a human lymphoid cell line have reported that continuous exposure to ARA-C up to 40 hours produces a significant accumulation of cells in S phase (17).

After a 3-hour exposure to 20 μg/ml of ARA-C, which produced > 85% inhibition of DNA synthesis in the HL-60 leukemic cells, there was about 35% recovery at 4 hours and 90% recovery at 8 hours of DNA synthesis (table 1). These data indicate that the HL-60 leukemic cells can recover rapidly from inhibition of DNA synthesis by a "high-dose" exposure to ARA-C.

Rustum and Preisler (18) reported that in patients with acute myeloblastic leukemia, the half-life of ARA-CTP in leukemic cells was greater in patients who had a longer duration of remission than in those who had a short duration of remission. These investigators reported an ARA-CTP half-life in the range of 1–8 hours. For HL-60 leukemic cells, we estimated the half-life of ARA-CTP to be about 60 minutes (fig 3). Since ARA-CTP is the pharmacologically active form of the drug (15), the rapid catabolism of this nucleotide analog may permit the HL-60 leukemic cells to recover rapidly from the inhibitory effects of ARA-C during the interval between drug exposures. These data are in agreement with the rate of recovery of DNA synthesis after ARA-C exposure (table 1). It is also possible that other mechanisms exist in the cell that may play an important role in recovery, such as DNA repair, which may remove ARA-C from DNA.

The next phase of high-dose ARA-C therapy for the treatment of acute leukemia should be directed towards optimization of the dose schedule. The factors discussed here should be taken into consideration in the design of new experimental protocols.

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Responses of RIF Tumors to Heat and Drugs: Dependence on Tumor Size[1,2]

Ding-Jiu Li[3,4] and George M. Hahn[3,*]

The effects of hyperthermia and drugs on RIF tumors of three sizes were studied in C_3H mice. The animals were treated with bleomycin, carmustine, or a combination of both drugs and with or without concomitant localized hyperthermia. The hyperthermia dose was 44°C for 30 minutes for each treatment. Each animal was given two treatments, 7 days apart. The tumors were classified according to approximate size: 0.1, 0.3, or 0.7 cm^3. Among the animals bearing the smaller tumors, no cures were observed. Growth delay measurements indicated that heat and drugs had approximately additive effects. Several cures were seen among the animals bearing medium- and large-sized tumors. Our results indicate that medium and large tumors may respond better to the combination treatment than small tumors.
[Cancer Treat Rep 68:1149–1151, 1984]

Enhancement of drug cytotoxicity by heat has been demonstrated in many studies involving cultured cells and murine tumors (1). For example, exposure of RIF tumors to carmustine (10 mg/kg) and heat (43°C, 30 minutes) three times resulted in a tumor doubling time of 35 days if the treatments were given simultaneously, but only 20 days if heat and drug administration were separated by 24 hours. The latter result reflected additive delays caused by heat or to drug individually.

In a different vein, two studies have recently indicated that large tumors may respond more favorably to the combination of heat and x-irradiation than small tumors (2,3). This latter finding is of considerable potential interest, since it is axiomatic among radiotherapists that large tumors are much more difficult to treat than small tumors. This feeling is also often expressed by chemotherapists who suggest that in many regions of large tumors, drug concentrations do not reach adequate cytotoxic levels and, therefore, therapy against these large tumors is ineffective.

We have undertaken a study to see if combined treatment of hyperthermia and chemotherapy could improve the response of large tumors. We have used the RIF tumor (4) to look at the interaction of heat and two chemotherapeutic agents in tumors of well-defined sizes. Our findings are that medium and large tumors may benefit more from combination treatments involving hyperthermia and chemotherapy than small tumors.

MATERIALS AND METHODS

The RIF tumor is syngeneic in C_3H mice. It has been described as being minimally or nonantigenic (4). For tumor initiation, 2×10^5 cells were implanted into the flanks of C_3H mice (age, 12–16 weeks). Details of the implantation technique have been described by Marmor et al (5).

Treatment was started 2 weeks after mice were injected. At that time, tumors were categorized into three groups: those with mean diameters of 4.8 mm (range, 4.3–5.7), about 0.1 cm^3 in volume, were classified as "small" tumors; those with mean diameters of 7.5 mm (range, 7.1–8.2), 0.3 cm^3 in volume, were classified as "medium" tumors; and those with mean diameters of 9.5 mm (range, 9.0–10.0), 0.7 cm^3 in volume, were classified as "large" tumors.

The hyperthermia "dose" was 44°C for 30 minutes for each treatment. Each animal was given two treatments, 7 days apart. Drug doses were: 7.5 mg/kg of body weight for bleomycin and 10.0 mg/kg of body weight for carmustine. The drugs were given ip 30 minutes before the onset of the hyperthermia treatment. Details of our method of radio frequency heating and of thermometry have been described (5,6).

Tumor cure and tumor doubling time were used as the experimental endpoints. Tumor cure is defined as the absence of regrowth of the tumor by 30 days after treatment (6); tumor doubling time is defined as the time re-

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TABLE 1.—Cures of RIF tumors treated by hyperthermia and chemotherapy

	Small tumors	Medium tumors	Large tumors
No treatment	0/12	0/12	0/10
Bleomycin	0/12	0/12	0/10
Carmustine	0/12	0/12	0/10
Bleomycin and carmustine	0/12	0/12	0/10
Hyperthermia	0/11	1/10	0/13
Bleomycin and hyperthermia	0/12	5/11*	3/11
Carmustine and hyperthermia	0/11	3/12	6/12*
Bleomycin, carmustine, and hyperthermia	0/12	4/11*	6/17*
Totals (combined treatments)	0/35	12/34†	15/40†

*Significantly different from "small" tumor values ($P < 0.1$; Student's t test).
†Significantly different from "small" tumor values ($P < 0.01$; Student's t test).

quired for doubling the diameter of the tumor (as measured at the time of treatment). To include tumor cures in the doubling time calculations, we assigned to cured tumors the longest doubling time observed in noncured animals from the same group. We define tumor growth delay as the time required for doubling the diameter of a treated tumor minus the average doubling time of untreated tumors of similar size.

RESULTS

Table 1 shows that treatments with either heat or drugs alone very rarely effected tumor cures (one of 136 mice). Combined hyperthermia and drug treatments were more effective (27 of 109). Segregating the latter by tumor size demonstrates that the combined treatment was much more effective against medium or large tumors (27

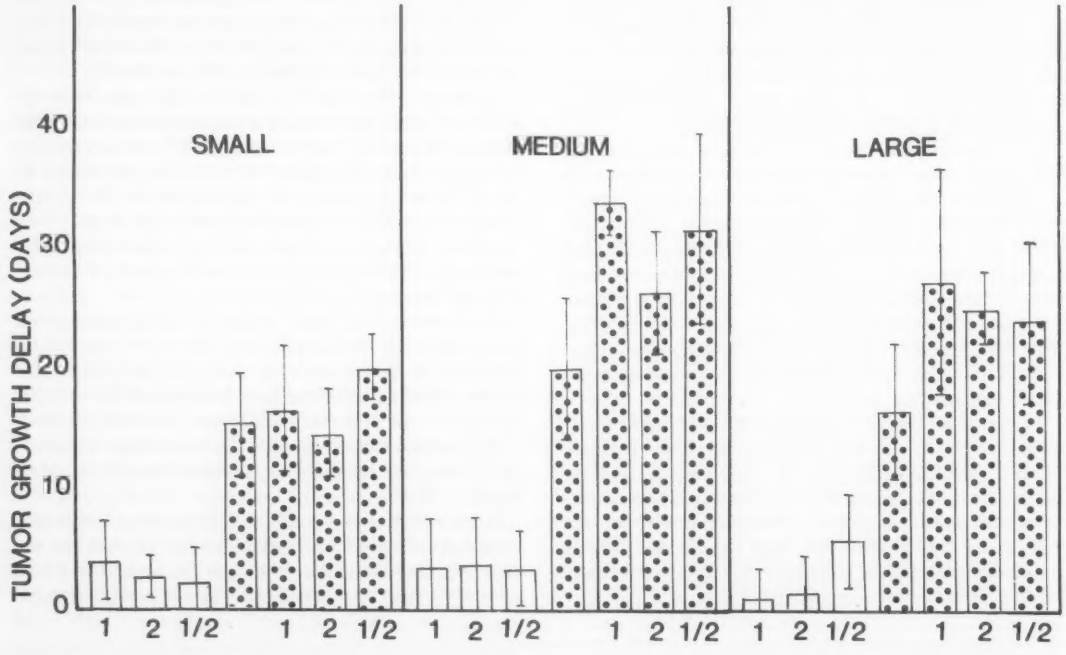

FIGURE 1.—Growth delay for RIF tumors in C_3H mice treated with carmustine (1), bleomycin (2), or both (1/2), with or without hyperthermia. All treatments involving hyperthermia are shown as stippled areas. The error bars represent ± SD of the mean. The No. of tumors per treatment is shown in table 1. Untreated tumor doubling times were 8.7 ± 1.0 for "small," 11.3 ± 2.5 for "medium," and 15.4 ± 2.1 for "large" tumors.

of 74) than against small tumors (none of 35).

Much the same result was obtained from the growth delay data (fig 1). This figure shows first of all that heat alone is far more effective against RIF tumors than either bleomycin, carmustine, or the combination of the two drugs. The figure also shows that the combination of heat and drugs induces greater growth delay in medium or large tumors than in small tumors and that such treatments are more effective than individual treatments.

DISCUSSION

The data we have presented show that murine tumors exposed to the combination of heat and drug respond in a manner modulated by tumor size. Large tumors tend to respond more favorably than small tumors. In this respect, our data are similar to those reported for x-irradiation and heat (2). There are several explanations that might account for what seems at first glance to be a paradoxical result. Low pH, nutritional deficiencies, and hypoxia might individually or in combination enhance drug action at elevated temperatures. Furthermore, heat and drugs may have combined effect against the vascular system of large tumors. Vascular effects are believed to be of considerable importance in determining the fate of heated tumors. Each of these hypotheses can be tested, and we are currently doing so. It is of course always hazardous to attempt to compare mouse data with clinical data. This is also true in the case of hyperthermia: differences in the dimensions between human and murine tumors are particularly troublesome. The data of Dewhirst et al (2), however, pertain to the treatment of spontaneous tumors in dogs and cats, while Manning et al (3) report on clinical findings. Yet, both groups of authors found results consistent with those reported here. We therefore feel justified in suggesting that our results do have treatment relevance. Localized hyperthermia given during a course of chemotherapy might be particularly effective against specific large tumors that prove refractory to drug treatment alone.

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Protection of Normal Hematopoietic Stem Cells From the Toxicity of Purine Base Analogs: In Vivo Application[1,2]

Joshua Epstein* and Harvey D. Preisler[3,4]

Normal murine bone marrow cells were incubated with adenosine (100 μM) for 2 hrs followed by the addition of 6-thioguanine (6-TG) (0.3 and 1.0 μg/ml) for 1, 24, and 48 hrs, and the survival of the committed stem cells (CFUc) and pluripotential stem cells (CFUs) was compared to the survival of CFUc and CFUs which were similarly incubated without adenosine. When 0.3 μg/ml of 6-TG was used, preincubation with adenosine increased the survival of CFUc from 25% to 80% and from 14% to 36% for 24- and 48-hr incubations, respectively ($P \leq 0.005$). For 1 μg/ml of 6-TG, pretreatment with adenosine increased survival from from 11% to 69% and from 5% to 24%, respectively, for 24- and 48-hr incubations ($P \leq 0.0005$). Adenosine had a similar effect on CFUs ($P \leq 0.03$). This effect was not seen when equimolar concentrations of hypoxanthine, inosine, or adenine were substituted for adenosine. Inoculation of adenosine into mice (500 mg/kg of body weight) 1 hr prior to injection of lethal doses of 6-TG (50 mg/kg) increased the survival of the mice from 10% to 100% and from 20% to 80%, respectively, using two administration schedules. This effect of adenosine, which, as has been previously shown, is the result of the depletion of cellular phosphoribosyl pyrophosphate pools, is potentially useful for the selective protection of normal hematopoietic cells from the toxicity of purine analogs which require activation via a phosphoribosyl pyrophosphate-dependent reaction, while therapeutic effects are produced against malignant tissue. [Cancer Treat Rp 68:1153–1156, 1984]

While the most common mechanism of cellular resistance to the purine analogs 6-thioguanine (6-TG) and 6-mercaptopurine (6-MP) is a deficiency in the phosphoribosylating enzyme HGPRT (1,2), we have reported that intracellular levels of phosphoribosyl pyrophosphate (PRPP), which are too low to permit the phosphoribosylation reaction to be carried out at a significant rate, confer on the cells resistance to 6-TG despite the presence of the HGPRT (3,4). Debatisse and Buttin (5) have reported that addition of adenosine to the medium rendered the tissue culture cells resistant to 6-TG and 6-MP. This effect of adenosine is exerted through the depletion of the cellular PRPP pools. The high levels of adenine nucleotides achieved produce feedback inhibition of the PRPP synthetase (5,6). Recently, we have demonstrated that this approach selectively protects normal cells, but not cells derived from hepatomas and lymphomas of the rat, against the toxicity of 6-TG and 6-MP (7).

A major limitation in the use of 6-TG, 6-MP, and adenine analogs in a clinical setting is their profound toxicity to normal hematopoietic stem cells. We have, therefore, tested the ability of adenosine to protect normal murine hematopoietic stem cells in vitro and in vivo from the toxicity of 6-TG and to protect animals from lethal doses of the drug.

MATERIALS AND METHODS

In vitro drug treatment.—Bone marrow cells were flushed out of the femurs of 8–12-week-old RFM/N mice and the rbcs lysed using ammonium chloride as previously described (8). Wbc counts (3×10^3) were recovered from each animal. The cells were suspended at 2×10^5/ml in RPMI-1640 medium supplemented with horse serum (10%) which contained no detectable adenosine deaminase activity. When indicated, adenosine was added and the cultures were incubated for 2 hrs at 37°C in a humidified atmosphere containing 7.5% CO_2 in air.

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TABLE 1.—Protection of normal CFUc from 6-TG toxicity: % survival of CFUc*

6-TG (μg/ml)	Adenosine (μM) 0	50	100
0	178 ± 22	144 ± 30	145 ± 38
5	72 ± 9	97 ± 20†	121 ± 8‡
10	72 ± 4	105 ± 10†	118 ± 4†
20	66 ± 7	108 ± 11‡	104 ± 7†
30	68 ± 10	113 ± 8†	108 ± 4‡

*Survival of 6-TG-treated cells is expressed as % of colonies recovered compared to the controls at the top of each column. Results for 6-TG-treated cells are expressed as the mean ± SE for 3 experiments, each carried out in 5 replicates.

†Statistically significantly higher at the $P = 0.01$ level than cells that were not preincubated with adenosine.

‡Significant at the $P = 0.05$ level.

6-TG was added to the cultures and the incubation was continued as described. When incubated for > 3 hrs, the cultures were supplemented with uridine as a source of pyrimidines, which is essential for the survival of the cells (7). Uridine was used at 20 μM, which was double the minimal concentration that assured survival of control cultures to which 6-TG was not added. Following incubation the cultures were washed free of the drugs (8) and survival of the committed stem cells (CFUc) and pluripotential hematopoietic stem cells (CFUs) was assessed.

CFUc assay.—Drug-treated cells were suspended in RPMI-1640 medium containing 20% heat-inactivated fetal calf serum, 10% colony-stimulating activity (9), and 0.3% agar at 5 × 10^4/ml. Aliquots (1-ml) were plated in 35-mm plates in quintuplicate. The plates were incubated at 37°C in a humidified atmosphere containing 7.5% CO_2 in air for 7 days. Colonies containing at least 50 cells were scored using an inverted microscope.

CFUs assay.—Eight 12-week-old mice were irradiated with 850 R of x-irradiation, and 2 hrs later inoculated with 0.2 ml of phosphate-buffered saline containing 2 × 10^4 marrow cells. Ten days later the mice were killed by cervical dislocation; their spleens were removed and fixed, and the number of colonies on the surface was scored (10). Five mice were used for each treatment group.

In vivo studies.—Eight 12-week-old animals were injected ip with adenosine alone, adenosine plus 6-TG, or adenosine followed by 6-TG. The doses of 6-TG and schedules of administration were adjusted to produce 80%–90% death rates when given alone.

Statistical methods.—Results of the CFUc and CFUs assays were analyzed using the Mann-Whitney test and/or Student's t test. Survival data were analyzed using the Fisher exact test (10). All statistical analyses were done using the Institute's mainframe computer.

RESULTS

In vitro.—The effects of 1-hr incubation with 6-TG on normal murine CFUc and the protective effect of adenosine are shown in table 1. Under the experimental conditions 6-TG was not very toxic to the normal murine CFUc, reducing their numbers by only one-third. In this case concentrations as high as 30 μg/ml were employed. No dose-response relationship was discernible. Adenosine by itself had little or no effect on the CFUc. Two hours' incubation with adenosine prior to the addition of 6-TG to the cultures resulted in recovery of colonies comparable to controls. Even though 6-TG alone reduced clonogenicity by only one-third, the protective effect of preincubation with adenosine was statistically significant at the $P \leq 0.05$ level (for cells incubated with 20 μg/ml of 6-TG alone vs 50 μM of 6-TG + adenosine and 30 μg/ml of 6-TG vs 100 μM of 6-TG + adenosine) and significant at the $P \leq 0.01$ level for the other five treatment groups. The ability of adenosine to protect CFUc from the toxicity produced by exposures to 6-TG for 24 and 48 hrs is demonstrated in table 2. In these experiments, uridine had to be added to the cultures to prevent a sharp decline in the survival of CFUc and CFUs, as described in the Materials and Methods section. Incubation with uridine alone (20 μM) had no effect on the CFUc or CFUs. Incubation with adenosine and uridine resulted in a loss of

TABLE 2.—Protection of CFUc from the effects of 24- and 48-hr exposure to 6-TG

Adenosine (μM)	Uridine (μM)	6-TG (μg/ml)	CFUc (mean ± SE) % of control* 24-hr	48-hr
0	0	0	100	100
0	20	0	98 ± 4	102 ± 8
100	20	0	73 ± 10	64 ± 8
0	20	0.3	25 ± 7	14 ± 1
0	20	1.0	11 ± 1	5 ± 1
100	20	0.3	80 ± 3†	36 ± 1†
100	20	1.0	69 ± 14†	29 ± 2†

*Results of the 24-hr experiments are expressed as the mean ± SE of the means of 3 experiments carried out in at least triplicates. Results for the 48-hr exposure are the mean ± SE of 1 experiment carried out in triplicate and are presented for comparison only.

†Protective effect of preincubation with adenosine was significant at the $P \leq 0.005$ level.

TABLE 3.—Protection of CFUs from 24-hr exposure to 6-TG

Adenosine (μM)	Uridine (μM)	6-TG (μg/ml)	CFUs (mean ± SE)	
			Experiment 1	Experiment 2
0	0	0	14.0 ± 1.8	18.8 ± 2.5
100	20	0	ND*	18.3 ± 2.3
0	20	0.3	4.6 ± 1.6	2.5 ± 0.9
0	20	1.0	2.6 ± 0.7	ND
100	20	0.3	18 ± 1.1†	8.8 ± 1.8†
100	20	1.0	6.5 ± 0.5†	ND

*ND = experiment was not done.
†Protective effect of preincubation with adenosine was significant at the $P \leq 0.03$ level.

27%–46% of CFUc for 24- and 48-hr incubations, respectively. Incubation with 0.3 μg/ml of 6-TG resulted in the killing of 75%–86% of the CFUc. Incubation with adenosine prior to exposure to 6-TG increased the survival of CFUc to 80% and 36% for 24- and 48-hr incubations, respectively. When 1 μg/ml of 6-TG was used, preincubation with adenosine increased the survival of CFUc from 11% to 69% and from 5% to 24%, respectively, for 24- and 48-hr incubations. The protective effect of adenosine on the CFUc was significant at the $P \leq 0.0005$ level for 24-hr exposures to 6-TG and at the $P \leq 0.0004$ level for 48-hr exposure to the drug. Similarly, the presence of adenosine in the culture medium significantly ($P < 0.03$) increased the survival of the normal murine stem cells (table 3). In several experiments inosine, hypoxanthine, or adenine was substituted for adenosine. None of these protected the CFUc or the CFUs from the effects of 6-TG. In all of the experiments performed, the protective effect of adenosine was best expressed when incubation with adenosine preceded the exposure to 6-TG. This is in accordance with our earlier report (7). Under the conditions described, the addition of 0.1 mM pentostatin to the incubation, together with adenosine to prevent deamination of adenosine, had no effect on the survival of the stem cells (results not shown).

In vivo.—Preliminary experiments showed that adenosine alone given ip twice daily for up to 5 days in doses of up to 500 mg/kg of body weight had no effect on the survival of the mice (results not shown). The only effects observed were temporary changes in animal behavior, expressed as slower reaction to stimulus immediately following the administration of adenosine at the higher doses. This effect disappeared within 15 mins after the injection. As a result of these observations it was decided to use the highest dose of adenosine possible, limited only by its solubility. The results of two experiments using two schedules of 6-TG administration are summarized in table 4. While 6-TG alone was lethal to 80%–90% of the animals, injection of adenosine 1 hr prior to 6-TG resulted in the survival of 80%–100% of the animals ($P < 0.003$, Fisher exact test). All of the control mice inoculated with only adenosine and saline survived normally. These effects were seen only when adenosine was administered prior to 6-TG as in the in vitro experiments. Equimolar doses of inosine failed to produce a similar effect.

DISCUSSION

Selective protection of normal cells from cytotoxic agents has been suggested as a potential approach to increasing the antitumor efficacy of cancer therapy (7). Adenosine has been reported to protect cell lines from the toxicity of 6-TG and 6-MP by reducing PRPP levels to the point where drugs such as these are not activated (5). Under appropriate conditions this effect could be directed towards the selective protection of cell lines of normal

TABLE 4.—Protection of normal RF mice from the toxicity of 6-TG

6-TG	Adenosine	Median survival in days (range)	Mortality (No. of mice dead/No. in group)
50 mg/kg, Days 1 and 2	None	9 (6–11)	8/10
50 mg/kg, Days 1 and 2	500 mg/kg, 1 hr prior to 6-TG	—	0/10*
50 mg/kg, Days 1 and 4	None	11 (8–16)	9/10
50 mg/kg, Days 1 and 4	500 mg/kg	— (8–16)	2/10†

*Significant at the $P = 0.0004$ level.
†$P = 0.003$.

origin, while cell lines derived from tumors and capable of forming tumors upon inoculation into normal recipients remained sensitive to the drugs (7). The presence of 2-pentostatin, the tight-binding inhibitor of adenosine deaminase, had no effect on the results, in accordance with findings with tissue culture cell lines where high concentrations of adenosine were used. This indicates that deamination of adenosine is not required for the protective effect to be exerted, and that the availability of adenosine was sufficient to sustain the protective effect for the duration of the presence of 6-TG. Inclusion of adenosine deaminase inhibitors is likely to reduce the selectivity of this phenomenon, which in part results from differences of the ratios of adenosine deaminase:adenosine kinase activities between cells. Our in vitro studies demonstrate that this phenomenon, which had heretofore been demonstrated only in established cell lines, is reproducible in primary culture of murine bone marrow stem cells using short and/or long exposures to the drugs. Pretreatment with adenosine was also effective in protecting the normal hematopoietic stem cells from 6-TG. No severe adverse reactions to the high doses of adenosine used in this study were noticed. Slowness of response to external stimulus was apparent for short periods following administration, possibly an expression of the agent's vasodilatory effect, but long-term toxicity was not produced. This may reflect the rapid removal of adenosine from the circulation by the endothelium (11) so that high adenosine levels were not maintained for long periods of time. On the other hand, the adenosine levels were clearly maintained long enough to allow sufficient accumulation of adenine nucleotides by the bone marrow stem cells to produce the desired effect. Since 6-TG is rapidly cleared from the circulation and is undetectable 60 mins after ip administration, it is not necessary to maintain suppression of PRPP for long periods of time (12). Further studies are required to establish conditions in vivo for the selective protection of normal tissues while therapeutic effects are produced against neoplastic tissues. This approach also has potential for ex vivo clearing of bone marrow from tumor cells for autologous marrow transplantation.

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Cisplatin, Vinblastine, and Bleomycin (CVB) Therapy for Relapsed Disseminated Neuroblastoma[1]

Bruce Bostrom,[2,*] William G. Woods,[2] Norma K. C. Ramsay,[2] William Krivit,[2] Peter Levine,[3] and Mark E. Nesbit, Jr[2]

The use of cisplatin, vinblastine, and bleomycin (CVB) combination chemotherapy in patients with disseminated neuroblastoma forms the basis of this report.

PATIENTS AND METHODS

Five patients with Evans stage IV neuroblastoma were treated from July 1981 to July 1983 with 21-day cycles of CVB (table 1). After a maximum dose of 270 mg/m^2 of bleomycin, therapy was continued with cisplatin and vinblastine alone in patients showing a response. Patient characteristics, including age at diagnosis, sex, prior therapy, duration of prior therapy, and sites of disease involvement, are listed in table 1. A cross-sectional area of lesions estimated by computerized tomography, bone marrow trephine biopsies, and urinary catecholamines was used to assess response. Response was defined as follows: *partial response*—a $\geq$ 50% decrease in all measurable lesions and tumor markers, with no new lesions or disease progression for 4 months; and *stable disease*—a $<$ 50% decrease, with no new lesions or disease progression for 4 months.

RESULTS

Response, duration of response in months, and survival in months are listed in table 1. Patient 1, who initially had a good partial response with a $>$ 90% reduction in a large abdominal mass, is now disease-free after residual disease was resected from the abdomen and liver. Pathologic examination of the surgical specimens showed large areas of fibrosis with minimal residual differentiating neuroblastoma. Patients 2-5 developed progressive disease after showing responses as listed. Patients 2-4 have died with progressive neuroblastoma.

Toxicity

All patients had audiograms, and four (Patients 1, 3, 4, and 5) developed a high-frequency hearing loss after four, five, nine, and five cycles of therapy, respectively. Only one patient (No. 3) was symptomatic and required a hearing aid. Two patients (Nos. 1 and 5) developed a decrease in creatinine clearance to 63 and 13 ml/1.73 m^2/minute, respectively, after nine and eight cycles of therapy, respectively. The creatinine clearance of Patient 1 returned to normal 6 months after therapy was discontinued. The other three patients had normal creatinine clearances after 4, 5, and 6 months of therapy.

Asymptomatic pulmonary infiltrates were documented in one patient (No. 1) after five cycles of therapy which resolved within 2 weeks after stopping bleomycin. One patient (No. 5) developed pulmonary nodules after 6 cycles of therapy that was not preceded by a decrease in pulmonary function tests. Biopsy of the nodules exhibited fibrosis secondary to bleomycin toxicity. Two other patients (Nos. 2 and 4) had serial pulmonary function studies. One patient (No. 2) had an asymptomatic decrease in pulmonary function studies following six cycles of therapy.

Bone marrow suppression was minimal in all patients. Patients 2 and 5 presented with pancytopenia due to extensive metastatic disease in the bone marrow and had normalization of blood cell counts during therapy due to a reduction of bone marrow neuroblastoma.

Other toxic effects included Raynaud's phenomenon during cold exposure in Patient 1, transient hypertension during vinblastine infusion in Patient 3, and hypomagnesemia (serum Mg, 0.8 mg/dl) in Patient 2.

DISCUSSION

Prior use of CVB for the treatment of disseminated neuroblastoma has been limited. Cisplatin alone has led

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TABLE 1.—Patient characteristics

Patient No.	Age (yrs)/ sex	Prior therapy* (mos)	Involved sites†	CVB cycles‡	Response	Duration § of response/ survival (mos)	Urinary catecholamines¶ (pre/nadir)
1	3/F	None	Abd; L; N	13	Partial/NED**	27+/27+	HVA (50.8††/9.8) VMA (10.0/5.1)
2	16/M	COD (2)	Abd; BM; L; N; M; B; IC	6	Partial	5/7	HVA (9.7/6.0) VMA (13.1††/8.2)
3	3/M	COD; CAV; Rad; Surg (24)	Abd; BM; L; N; M	5	Stable disease	5/7	VMA (66††/36††)
4	5/M	COD; Surg; BMT (12)	Abd; BM; N	4	Partial	4/5	Normal
5	5/M	COD; Surg (5)	Abd; BM; B; L; N; IC	8	Partial	7/9+	HVA (373††/15††) VMA (293††/15††)

*COD = cyclophosphamide, vincristine, and dacarbazine; CAV = cisplatin, doxorubicin, and teniposide; Rad = local radiotherapy; Surg = surgical resection of abdominal tumor; and BMT = allogeneic marrow transplant following vincristine, cyclophosphamide, melphalan, and total-body irradiation.

†Abd = abdominal tumor, L = liver, N = lymph nodes, BM = bone marrow, M = mediastinum, B = bone, and IC = intracranial.

‡CVB = cisplatin, 20 mg/m^2/day on Days 1–5 (10 mg/m^2/day if creatinine clearance < 75 ml/1.73 m^2/min); vinblastine, 4.5 mg/m^2/day on Days 1 and 2; and bleomycin, 15 mg/m^2/day on Days 2, 9, and 16 (iv push) (maximum dose, 270 mg/m^2). Patient 1 had bleomycin discontinued after 5 cycles because of pulmonary infiltrate.

§From start of CVB until disease progression.

¶HVA = μg of homovanillic acid/mg of creatinine; VMA = μg of vanillylmandelic acid/mg of creatinine except for Patient 3, where results are mg/24 hrs.

**Patient 1 was rendered disease-free with surgical resection following a > 90% reduction in abdominal tumor. NED = no evidence of disease.

††Elevated for age.

to two complete responses (17%) and five partial responses (42%) in 12 evaluable patients with relapsed neuroblastoma (1,2). When combined with teniposide in 43 evaluable patients with neuroblastoma refractory to conventional chemotherapy, cisplatin was associated with seven complete responses (16%) and 16 partial responses (37%) (3,4). Cisplatin has also been used in conjunction with doxorubicin in 15 patients with refractory neuroblastoma with two complete responses (18%) and one partial response (7%) (5).

Vinblastine has been used with continuous infusion bleomycin in six previously treated relapsed patients with no response (6). Vincristine plus bleomycin also was ineffective in 19 previously treated patients with progressive Evans stage IV neuroblastoma (7).

In the present series, four of five patients achieved partial responses with CVB. These results compare favorably to other cisplatin-containing regimens used in the treatment of refractory neuroblastoma. The addition of vinblastine and bleomycin, although possibly not effective alone in relapsed neuroblastoma, may give added effect with cisplatin because of recruitment of cells into the cell cycle by cisplatin, thereby increasing the activity of vinblastine and bleomycin (8).

In conclusion, the CVB combination warrants further evaluation in disseminated neuroblastoma as a salvage regimen for relapsed patients. If results are as promising as those in this study, CVB should be considered as part of combination chemotherapy for the initial treatment of metastatic neuroblastoma.

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Use of Cisplatin, Cyclophosphamide, Vincristine, and Doxorubicin for the Treatment of Non-Small Cell Lung Cancer[1,2]

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Forty-four evaluable patients with non-small cell carcinoma of the lung were treated with cisplatin, cyclophosphamide, vincristine, and doxorubicin. The overall response rate was 57%. The 16% who had complete response had a median survival of 81 weeks. [Cancer Treat Rep 68:1159–1161, 1984]

Lung cancer is the leading cause of cancer deaths in this country. Nearly 117,000 people died from this cancer in 1983 (1). Unfortunately, the overall 5-year survival of patients with non-small cell lung cancer ranges between 5% and 10% (1,2). Chemotherapy for non-small cell lung cancer has generally not resulted in substantial benefit to patients. Although consistent responses were observed when doxorubicin was used in combination therapy (3–6), the addition of cisplatin has resulted in responses from 38% to 50% (7–9). The use of vindesine and cisplatin was also encouraging; the longest duration of response occurred in those patients who received 120 mg/m^2 of cisplatin (10). We therefore chose to use cyclophosphamide, doxorubicin, cisplatin, and vincristine in place of the newer vinca alkaloid, vindesine.

MATERIALS AND METHODS

Forty-six patients with non-small cell lung cancer who had not been previously treated with chemotherapy and who were not surgical candidates were offered treatment with a Connecticut Oncology Association protocol. The patients were given 120 mg/m^2 iv of cisplatin over 20 minutes following iv hydration and 12.5 g of mannitol, 600 mg/m^2 iv of cyclophosphamide, 2 mg iv of vincristine, and 30 mg/m^2 iv of doxorubicin if they gave informed consent. Therapy was repeated every 4 weeks for three cycles and then extended every 6 weeks for 1 year or until relapse. Response was defined as follows: *complete response*—total clinical disappearance of all known tumors for > 2 months; *partial response*—a 50% reduction in the product of the two greatest diameters of all measurable lesions for at least 2 months. Repeat bronchoscopy or surgery was not done; chest x-rays, computerized tomographic scans, and other appropriate tests were used for evaluation of response. All patient charts and x-rays were evaluated by D. Lindgren and E. Cadman to verify responses.

RESULTS

The overall patient characteristics and results are shown in table 1. Of the 44 patients evaluable for a response (two patients died from extensive disease within 1 week of starting treatment), 16% had a complete response, 41% had a partial response, and 43% did not respond. The median survival for those achieving a complete response was 81 weeks. Those patients who were ambulatory (performance status 0 or 1 by Eastern Cooperative Oncology Group criteria) (11) did better. Of the 31 patients in this group, 23% had a complete response and 42% had a partial response. None of the 13 patients who were not ambulatory at the onset of therapy had a complete response; 38% had a partial response.

The toxic effects were significant: 56% of the patients had nausea and vomiting in spite of varied use of antiemetics and 24% were anorexic for at least 1 week. Eighty-seven percent of the patients had a reduction in wbc counts < 4500/mm^3. The median wbc count was 2000/mm^3 and the median platelet count was 50,000/mm^3 with a range from 6000 to 124,000/mm^3. Thirty of the patients had a > 2-g/dl reduction in their hemoglobin; 12 patients required rbc transfusions. Four patients had a rise in the serum creatinine > 2 mg/dl. In two patients with advanced disease, the renal failure occurred shortly after the first treatment. Two patients noted a slight reduction in hearing.

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TABLE 1.—Survival of 46 patients treated with cisplatin, cyclophosphamide, vincristine, and doxorubicin

Characteristic	Median survival (wks)			No. of patients	
	Responders	Nonresponders	Overall	Alive	In sample
All patients	57.8	28.8	40.3	19	46
Histology					
Squamous cell	66.5	37.9	43.4	7	18
Adenocarcinoma	42.4	25.0	39.7	10	20
Large cell	40.0	6.0	12.0	2	8
Extent of disease					
Extensive			38.0	17	38
Limited			41.7	2	8
Sex					
Male	46.4	29.8	40.3	12	32
Female	> 63	14.0	33.9	7	14
Prior treatment					
None or surgery			44.0	14	32
Radiotherapy			21.9	5	14
Performance status*					
0			81.0	3	5
1			46.1	15	26
2			18.0	1	5
3			10.0	0	10
0-1	72.4	39.3	60.3	18	31
2-3	18.5	13.0	14.5	1	15
Age in yrs					
30-39			> 25	3	4
40-49			> 63	4	6
50-59			26.2	4	13
60-69			34.2	6	18
70-79			41.0	2	5
Response†					
CR			81.0	4	7
PR			43.3	10	18
NR			29	5	19

*Eastern Cooperative Oncology Group criteria.

†2 patients died within 1 wk of therapy from extensive disease. CR = complete response, PR = partial response, and NR = no response.

DISCUSSION

This combination drug program appears to be of some use for the treatment of non-small cell carcinoma of the lung, although its use was associated with considerable toxicity. There is no obvious reason for the large number of patients with a high performance status. As expected, the ambulatory patients had the best response. Multiple investigators and hospitals participated in the study. Therefore, selection bias could be responsible in that the very ill patients might not have been offered or did not accept the therapy because of the potential side effects. However, without a controlled study, it is difficult to be certain that the median survival of the patients who achieved complete response was in fact due to therapy or simply because the patients were healthier. Further controlled and larger clinical trials are needed to define the role of combination therapy for this cancer; however, it is encouraging to document clinical complete responses in non-small cell cancer of the lung. This treatment program is currently being used at the Cancer Research Institute of the University of California, San Francisco, for all patients with nonresectable lung cancer, regardless of performance status.

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Doxorubicin and Cisplatin for Recurrent or Metastatic Squamous Cell Carcinoma of the Head and Neck[1,2]

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Thirty-six patients with recurrent or metastatic squamous cell carcinoma of the head and neck were treated with doxorubicin and cisplatin. The overall response rate (complete + partial) was 30%, with a median duration of response of 4 months. Median survival durations for responders and nonresponders were 15 and 4 months, respectively. Nausea, vomiting, and alopecia were common. Neither grade 4 myelosuppression nor irreversible renal failure was observed. [Cancer Treat Rep 68:1163–1165, 1984]

Cisplatin and doxorubicin were combined in a broad phase II trial because these agents had shown activity against a variety of tumors and because their major toxic effects were different (1,2). This regimen produced a 37% response rate and acceptable toxicity in 16 patients with squamous cell carcinomas of the head and neck (3). Based on these initial results and on the potential advantage of being able to give this regimen on an outpatient basis, this treatment was given to 20 additional patients. The results for all 36 patients are described in this report.

MATERIALS AND METHODS

Eligibility was limited to patients with histologically confirmed incurable squamous cell carcinoma of the head and neck. All patients must have recovered from the effects of previous irradiation or surgery and must have had no prior treatment with doxorubicin and cisplatin. Eligibility requirements included: measurable disease, Eastern Cooperative Oncology Group (ECOG) performance status < 4, wbc count > 4000/mm^3, platelet count > 100,000/mm^3, bilirubin < 1.5 mg/100 ml, SGOT < 60 IU, BUN < 25 mg/100 ml, serum creatinine < 1.5 mg/100 ml, creatinine clearance > 50 ml/minute, and no history of congestive heart failure or uncontrolled hypertension. Informed consent was always obtained.

Treatment consisted of doxorubicin at a dose of 50 mg/m^2 given as an iv bolus, followed by cisplatin at a dose of 50 mg/m^2 iv at a rate of 1 mg/minute. Each patient received 1 L of 0.2 normal saline prior to the cisplatin. Diuretics and posttreatment hydration were not given. The drugs were administered every 3 weeks on an outpatient basis. The total dose of doxorubicin was limited to 550 mg/m^2 and was reduced to 450 mg/m^2 in patients who had received prior thoracic irradiation.

The doses of doxorubicin and cisplatin were reduced by 50% if the wbc count was 3000–4000/mm^3 and the platelet count was 75,000–100,000/mm^3. The drugs were withheld if the leukocyte count was < 3000/mm^3 or the platelet count was < 75,000/mm^3. Cisplatin was not given if the BUN was > 25 mg/dl or the creatinine was > 1.5 mg/dl. Cisplatin was resumed at 75% of the initial dose at the start of the next cycle if the BUN was < 25 mg/dl and the creatinine was < 1.5 mg/dl. Responses were defined according to ECOG criteria (4).

RESULTS

Thirty-nine patients were entered in this study, and three were excluded for the following reasons: nonmeasurable disease (one patient) and doxorubicin deleted from the regimen (two). The median age of the group was 56 years (range, 20–81). Other patient characteristics are shown in table 1.

The overall response rate was 30%, with three complete and eight partial responses. Responses lasted from

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TABLE 1.—Response rate by entry characteristics

	No. of patients	
	Total	Responders (%)
Performance status (ECOG)		
0,1	15	5 (33)
2,3	21	6 (28)
Sex		
Male	26	9 (35)
Female	10	2 (20)
Site of metastatic disease		
Local-regional	26	7 (27)
Distant	3	0 (0)
Both	7	4 (57)
Previous radiation therapy		
Yes	34	10 (33)
No	2	1 (50)
Previous chemotherapy		
Yes	12	3 (25)
No	24	8 (33)
Primary site		
Larynx	8	1 (12.5)
Oral cavity	11	3 (27)
Oropharynx	7	2 (28)
Hypopharynx	3	1 (33)
Nasopharynx	4	2 (50)
Maxillary antrum	3	1 (33)

1 to 43 months, with a median duration of 4 months. Median survival durations for responders and for nonresponders were 15 and 4 months, respectively.

Seventeen patients were classified as having a minor protocol violation because of a 25% dose reduction of doxorubicin. Response rates were 37% for patients who received full doses and 24% for patients who received reduced doses of doxorubicin. This difference is not statistically significant (chi-square $\alpha = 0.6148$).

Nausea, vomiting, and alopecia occurred in virtually all patients. Two patients experienced moderate stomatitis. Paresthesias involving the extremities occurred in one patient. Clinically detectable hearing loss was not observed, although audiograms were not routinely done.

The following levels of toxicity were not observed: granulocyte count $< 1000/mm^3$, platelet count $< 100,000/mm^3$, and serum creatinine > 2 mg/dl. Nadir cell counts were not done. A decrease of > 2 g of hemoglobin not apparently related to bleeding occurred in eight patients. No cardiac toxicity was observed.

DISCUSSION

Chemotherapy produces higher response rates in previously untreated patients who have advanced head and neck cancer (5). Because doxorubicin and cisplatin were used as secondary treatment in our patients, our results are compared only to studies in which chemotherapy was not used as primary treatment.

The 30% response rate and median response duration of 4 months observed in our patients are virtually identical (27%–39%) to the results from trials using higher doses of cisplatin as a single agent (6–8). Amer et al (9) have identified performance status and prior treatment with chemotherapy as significant prognostic factors in patients with head and neck cancer. Comparing these variables in our patients and in the patients from the cisplatin trials shows that 33% of our patients had received prior chemotherapy versus 77% (6), 22% (7), and 0% (8). ECOG performance status was 2–3 in 60% of our patients compared to 33% in one of the cisplatin single-agent trials (8). This variable was not described in the other studies (6,7). The similar response rates for our regimen compared to higher doses of cisplatin alone may be explained by differences in patient populations.

Because the dose of cisplatin in the single-agent trials (6–8) was 60%–140% higher than the dose used in our study, the similar results might be explained by the existence of a relatively steep dose-response curve for cisplatin in head and neck cancer. A randomized trial evaluating the dose-response relationship of cisplatin in head and neck cancer is in progress (10).

The reduction of the doxorubicin dose in 47% of our patients may also have contributed to the relatively low response rate. However, this difference was not statistically significant.

The same dose and schedule of cisplatin (50 mg/m^2) combined with bleomycin and methotrexate (11,12) have produced response rates of 61% and 19%. A slightly lower dose of cisplatin (40 mg/m^2) combined with cyclophosphamide and doxorubicin has resulted in a 64% response rate (13). Evaluation of these factors in the studies which produced higher response rates (11,13) revealed that a performance status of 2–3 was observed in 45% of our patients versus 32% (11) and 6% (13) and that prior chemotherapy had been given to 33% of our patients versus 3.7% (11) and none (13). Distribution of performance status was virtually identical in the study which showed a lower response rate (12).

Although a randomized trial would be required to draw a definitive conclusion, it appears that the combination of doxorubicin and cisplatin at the doses used in our trial is not more effective than higher doses of cisplatin alone and may be less effective than other moderate-dose cisplatin combination regimens.

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Combination Chemotherapy for Metastatic Urinary Bladder Cancer With 5-FU, Vincristine, Bleomycin, Cyclophosphamide, Mitomycin, and Methotrexate[1]

Yoshinobu Kubota,[2,*] Taro Shuin,[2] Takeshi Miura,[2] Masatoshi Moriyama,[2] Shuji Fukushima,[3] Hiroshi Shiozaki,[4] and Hiroyuki Ooshima[2]

Twenty-two patients with advanced primary bladder cancer with metastatic lesions were treated with a combination of 5-FU, vincristine, bleomycin, mitomycin, and methotrexate. Complete and partial responses were achieved in two (9%) and five (23%) patients, respectively. The overall response rate was 32%. The major toxic effects were myelosuppression and mild gastrointestinal symptoms. [Cancer Treat Rep 68:1167–1168, 1984]

The prognosis of advanced urinary bladder cancer has been currently very poor. Reports which evaluate the efficiency of combination chemotherapy for metastatic bladder cancer have been particularly sparse (1).

The present paper reports the results of combination chemotherapy utilizing 5-FU, vincristine, bleomycin, cyclophosphamide, mitomycin, and methotrexate in the treatment of metastatic urinary bladder cancer.

PATIENTS AND METHODS

Twenty-two patients with primary urinary bladder cancer (16 males and six females, with the average age of 61.9 years [range, 45–77]) were selected. All of the patients had transitional cell carcinoma, with histological grading (TNM classification) of G3 in 21 patients and G2 in one patient, and metastatic lesions. Five patients had received prior tegafur treatment and the other 17 had not received any prior chemotherapy.

The chemotherapy regimen was carried out according to that of Kimura et al (2), except for the addition of methotrexate (table 1). In addition to the above, leucovorin at a dose equal to that of methotrexate was used as a "rescue" from methotrexate toxicity on Day 2, 6 hours after the methotrexate administration.

Patients were maintained on the treatment plan for at least two or more sequential courses, until manifestation of myelosuppression. The effect of the treatment was monitored by chest roentgenography, abdominal ultrasonography, iv pyelography, liver scintiscanning, and computerized axial tomography.

Evaluation of tumor regression was objectively defined as follows: *complete response (CR)*—the disappearance of all measurable tumor continuing for > 2 months; *partial response (PR)*—a > 50% decrease of total tumor volume; and *no response*—a < 50% regression of total tumor volume.

RESULTS

The response of metastatic lesions of bladder cancer to the present chemotherapy regimen was evaluated. CRs were achieved in two patients for 3 and 4 months. PRs were achieved in five patients. The overall response rate (CR + PR) was 32%. The remaining patients were judged as nonresponders, although three patients showed minor responses of metastatic lesions, one had a decrease of the pleural effusion, and two had a decrease of leg edema. Two patients had disease progression, even receiving the present combination chemotherapy. Almost all of the responses were observed within 2 weeks after starting the combination chemotherapy. The most obvious tumor regression usually occurred within 6 weeks; pain relief usually occurred within 1 week.

The average survival times of responders (CR + PR) and nonresponders were 6.9 ± 1.6 and 5.0 ± 3.9 (mean ± SD) months, respectively.

Various toxic effects were noted during the chemotherapy regimen. Mild gastrointestinal symptoms including nausea and vomiting on Days 1 and 2 appeared in all patients. One-half of the patients had bone marrow suppression (wbc count < 3000/mm^3 and platelet count

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TABLE 1.—Combination chemotherapy schedule for metastatic urinary bladder cancer

Drug (dose)	Day—						
	1	2	3	4	5	6	7
5-FU (200 mg/m^2 iv) or tegafur (400 mg/m^2 iv)	X	X	X	X	X	X	X
Vincristine (1 mg/m^2 iv)	X						
Bleomycin (15 mg/m^2 iv)		X					
Cyclophosphamide (200 mg/m^2 iv)			X				
Mitomycin (4 mg/m^2 iv)				X			
Methotrexate (10 mg/m^2 iv × 2)	X	X					

< 50,000/mm^3). Five patients had wbc count nadirs < 1000/mm^3 and two patients had platelet count nadirs < 10,000/mm^3, exhibiting temporary bleeding tendency. One of the above patients died of meningitis after three courses of the combination chemotherapy. Stomatitis was observed in six patients and spiking fever was observed in ten. Five of the patients had fever on Day 2. Another five patients had myelosuppression at the time of fever. In addition, gastrointestinal bleeding, alopecia, neuralgia, and ileus were observed separately.

DISCUSSION

For the present study, methotrexate was added to the chemotherapy regimen that was originally described by Kimura et al (2). The original regimen was developed for the treatment of lung cancer. To know the effect of the combination therapy on urinary bladder cancer, we selected 22 patients with advanced bladder cancer accompanying clinically measurable metastatic lesions for the present study.

The present result is not better than that previously achieved when cisplatin-containing combination chemotherapy was used to treat advanced bladder cancer with or without metastatic lesions (1,3). When 14 patients with metastatic lesions were treated with cisplatin alone, the result of five partial responders (36%) was comparable to that in the present study (4). Thus, the present combination chemotherapy has an antitumor effect comparable to the regimen using cisplatin.

The major toxic effect of the present chemotherapy regimen is myelosuppression. Although the myelosuppression can be controlled to some extent by isolating the patients, by administering antibiotics and gamma globulin, and by transfusing granulocytes and thrombocytes, it has been the major reason for discontinuing the present chemotherapy.

It appears most likely that the present regimen should be tried in patients with advanced bladder cancer to whom cisplatin is not applicable, as well as in those who had relapsed after a regimen including cisplatin.

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Acute Electrocardiographic Changes Induced by Amsacrine[1]

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The ECG effect of amsacrine (*m*-AMSA) was evaluated in 12 consecutive patients with leukemia. *m*-AMSA induced a significant prolongation of the Q-T interval (msecs, mean ± SE) before (448 ± 13) and 1 hour after (512 ± 12) treatment (P . 0.0001, paired t test), without concomitant changes in the P-R interval, QRS duration, and heart rate. This selective cardiotoxic effect appeared to be transient and was noted towards the end of the iv drug administration, but was not present 24 hours later. No cardiac arrhythmias were noted during continuous monitoring. Nevertheless, it is assumed that the prolongation of the Q-T interval may represent a state of increased vulnerability to rhythm disturbances. Special care should be taken to avoid factors that may prolong the Q-T interval (hypokalemia, ischemia, or premedication with phenothiazine) during the administration of *m*-AMSA. [Cancer Treat Rep 68:1169–1172, 1984]

Amsacrine (*m*-AMSA) is a new acridine derivative (1) that was introduced into clinical studies in 1977 (2). It has significant antitumor activity against acute leukemias and lymphomas (3–7) as well as against solid tumors (8,9). Myelosuppression is the major side effect of *m*-AMSA (10). Other side effects include nausea and vomiting, stomatitis, alopecia, local phlebitis, and hepatotoxicity (11). Sporadic reports of acute cardiac toxicity have recently been described. These cardiac effects included fatal ventricular fibrillation (7,12–15), supraventricular arrhythmias (7,12), and nonspecific ST-T wave changes (16); these were generally attributed to the presence of hypokalemia during treatment with the drug. This study was aimed to prospectively evaluate the incidence of rhythm disturbances as well as to look for an "arrhythmogenic" electrophysiological effect of *m*-AMSA.

PATIENTS AND METHODS

Twelve consecutive patients treated with *m*-AMSA were studied. There were nine men and three women between the ages of 16 and 38 years. Ten patients had refractory acute nonlymphoblastic leukemia (ANLL) and two had acute lymphoblastic leukemia (ALL). *m*-AMSA was given following failure of primary induction treatment or refractoriness to all conventional chemotherapy during the first or subsequent relapse. Previous treatment included a combination of cytarabine (Ara-C), thioguanine (TG), and daunorubicin for ANLL and doxorubicin, vincristine, prednisone, cyclophosphamide, and L-asparaginase for ALL.

Treatment protocol.—*m*-AMSA was given iv over 60–90 minutes. Two schedules were used: *schedule 1*—*m*-AMSA at a dose of 150 mg/m^2/day for 5 consecutive days given to six patients; and *schedule 2*—*m*-AMSA used in combination with Ara-C and TG at a dose of 90 mg/m^2 for 3 days, replacing daunorubicin in this schedule, given to the other six patients. Serum electrolytes, liver and renal functions, and blood cell counts were measured daily before and during treatment. *m*-AMSA was given only when the serum potassium level was > 4 mEq/L. Metoclopramide (Pramin), not phenothiazine, was used as an antiemetic agent.

ECG evaluation.—(*a*) A 12-lead ECG at a paper speed of 50 mm/second was recorded (Hewlett Packard No. 1511B ECG recorder) in every patient before and 1 hour after each iv infusion of *m*-AMSA. The following parameters were measured in all 12 leads recorded: interbeat interval, P-R interval, Q-T interval, and QRS duration (17). The Q-T interval was measured from the beginning of the Q wave to the intersection between the tangential line over the terminal limb of the T wave and the isoelectric line (18) and was corrected for heart rate (19). (*b*) A 48-hour ECG recording was performed in ten patients before, during, and after the first dose of *m*-AMSA using an Avionics Holter monitor (Los Angeles, CA).

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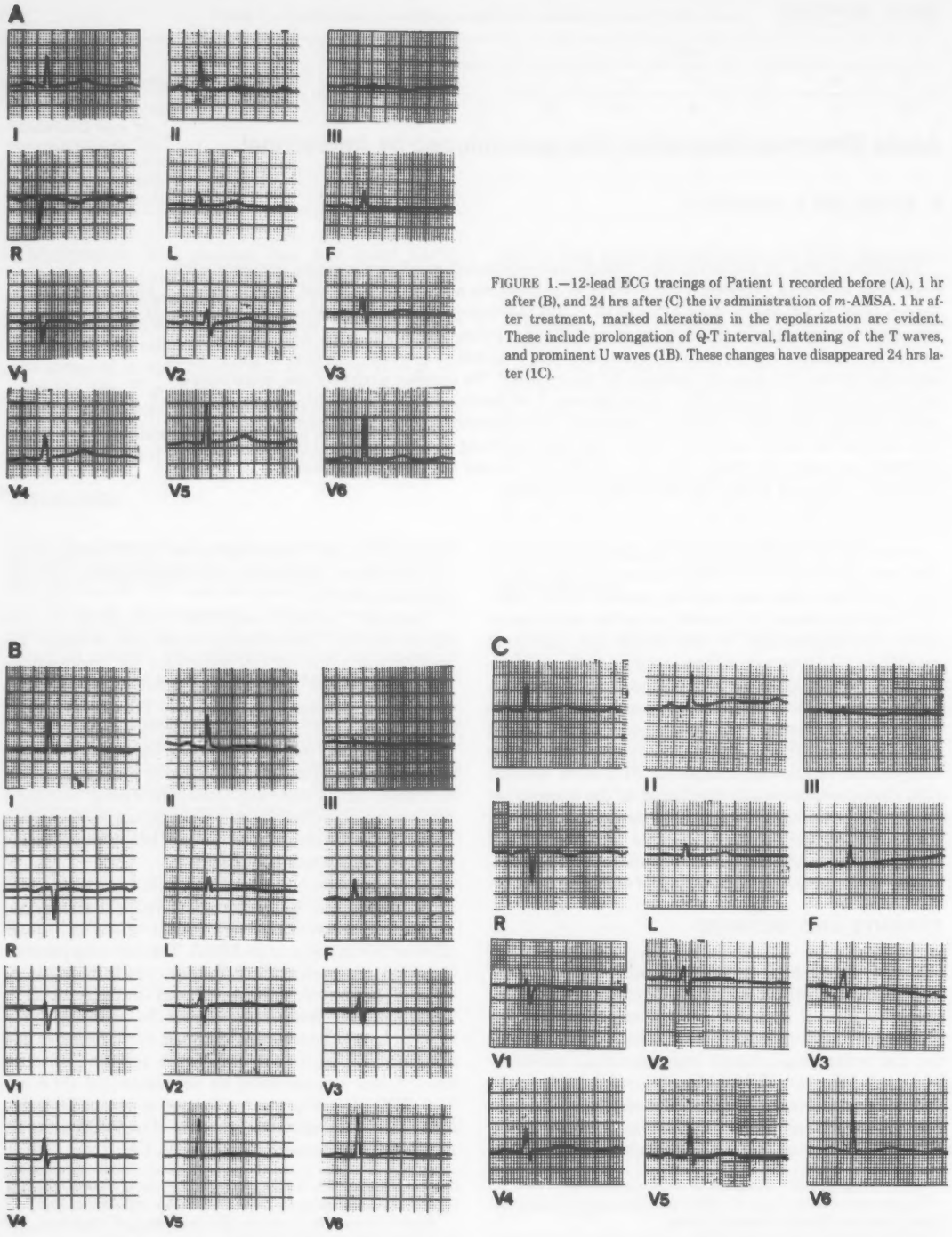

FIGURE 1.—12-lead ECG tracings of Patient 1 recorded before (A), 1 hr after (B), and 24 hrs after (C) the iv administration of *m*-AMSA. 1 hr after treatment, marked alterations in the repolarization are evident. These include prolongation of Q-T interval, flattening of the T waves, and prominent U waves (1B). These changes have disappeared 24 hrs later (1C).

RESULTS

Hematologic response.—Clinical improvement was noted in five of the 12 patients who received *m*-AMSA either as a single drug or in combination with Ara-C and TG: four with ANLL and one with ALL. Four of these patients achieved complete hematological remission that lasted 2–5 months, while the fifth patient had a partial response which lasted for 2 months. The remaining seven patients did not respond at all. These results are in accord with others (7).

Cardiac findings.—Acute alterations in the ST-T segment were observed in all the patients studied. A representative ECG tracing is shown in figure 1 and the Q-Tc values obtained from all ECG L_2 records are shown in figure 2. There was a significant prolongation of the Q-Tc interval (msecs, mean ± SE) before (448 ± 13) and 1 hour after (512 ± 12; $P < 0.001$, paired t test) drug infusion (fig 2). These findings were completely reversible 24 hours after treatment (Q-Tc, 434 ± 12 msecs), but recurred with repeated administration of the drug (fig 2). The prolongation of the Q-Tc interval was observed in all the 12 ECG leads, indicating a diffuse effect (table 1). There were no changes in the heart rate, P-R interval, and QRS duration. The serum potassium level was 4.6 ± 0.3 mEq/L in all the patients during *m*-AMSA administration. No cardiac dysrhythmias or changes in heart rate were recorded in ten of the patients undergoing continuous 48-hour ECG monitoring. No ECG changes were found in nine other patients with leukemia following an iv infusion of saline (three patients), Ara-C (three), and cyclophosphamide (three).

DISCUSSION

The present study describes a significant prolongation of the Q-T interval induced by iv administration of *m*-AMSA, while the P-R interval, QRS duration, and heart rate remained unaltered. This selective effect was transient, occurred repeatedly after readministration of the drug, and was not accompanied by any disturbances in cardiac rhythm. Prolongation of the Q-T interval may increase the vulnerable period of the myocardial tissue, thus facilitating the appearance of ventricular arrhythmias (20). Similarly, the initiation of atypical ventricu-

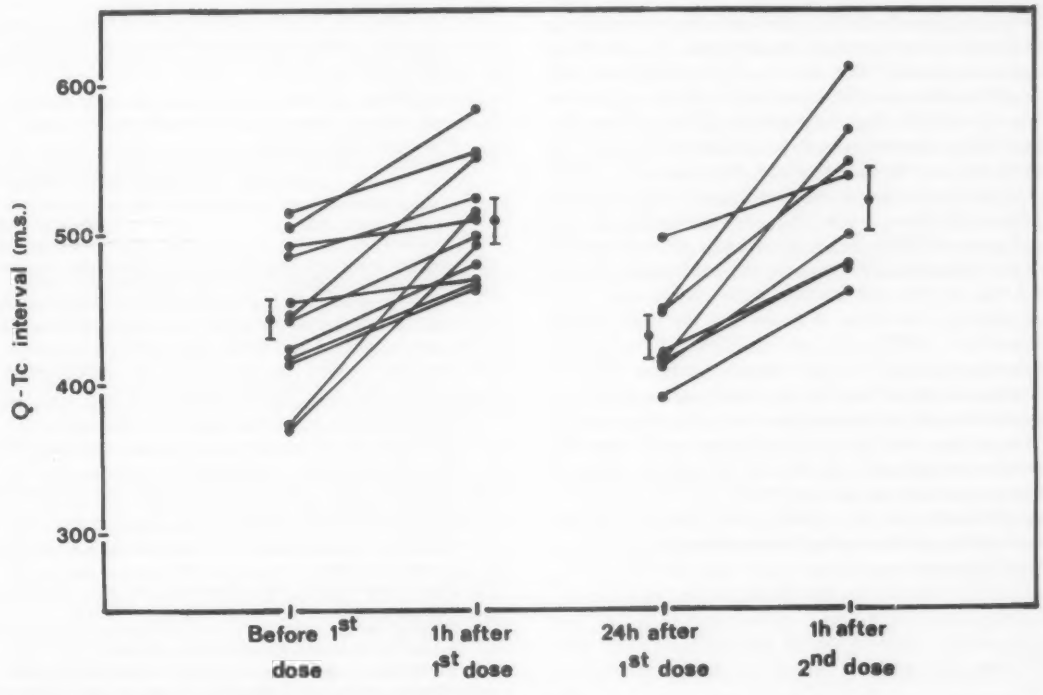

FIGURE 2.—Q-Tc values (msecs) measured from L_2 of the peripheral ECG before and 1 hr after iv administration of 2 consecutive doses of *m*-AMSA: there was a significant increase in the Q-Tc from 448 ± 13 msecs (mean ± SE) before to 512 ± 12 msecs (mean ± SE) after the 1st dose ($P < 0.0001$, paired t test). 24 hrs later the Q-Tc was shortened to 434 ± 12 msecs ($P < 0.000$ compared to that measured 1 hr after the 1st dose) and was increased again to 522 ± 20 msecs 1 hr after the administration of the 2nd dose ($P < 0.0001$).

TABLE 1.—Q-Tc duration (msecs) in all 12 ECG leads in 12 patients before and 1 hr after treatment with *m*-AMSA*

ECG lead	Q-Tc before *m*-AMSA (mean ± SE)	Q-Tc after *m*-AMSA (mean ± SE)
L_1	436 ± 18	520 ± 14
L_2	448 ± 13	512 ± 12
L_3	425 ± 9	492 ± 10
aVR	436 ± 12	507 ± 17
aVL	427 ± 10	491 ± 13
aVF	433 ± 11	498 ± 14
V_1	415 ± 12	482 ± 11
V_2	450 ± 14	495 ± 10
V_3	454 ± 12	501 ± 13
V_4	447 ± 9	504 ± 11
V_5	454 ± 13	510 ± 14
V_6	445 ± 10	498 ± 12

*Significant Q-Tc prolongation in all leads after treatment ($P < 0.01$, paired t test).

lar tachycardia ("Torsade de pointes") is attributed to impaired ventricular repolarization, reflected by prolongation of the Q-T interval (21). None of our patients developed cardiac dysrhythmias despite the Q-T prolongation, as could be seen from 48-hour ECG tape recordings performed during the administration of the drug. Nevertheless, fatal arrhythmias have been described by others during treatment with *m*-AMSA and these were mostly attributed to concomitant hypokalemia. An additional factor could have been the use of premedication with phenothiazines as antiemetics, before the iv administration of *m*-AMSA. Both hypokalemia (22) and phenothiazines (23) are known to cause prolongation of the Q-Tc interval and may have augmented the effect of *m*-AMSA on the repolarization process, resulting in ventricular fibrillation. We have avoided the use of phenothiazines and only gave *m*-AMSA in the presence of normal levels of serum potassium. This may be the explanation for the fact that rhythm disturbances were not encountered in our patients. Therefore, it seems that the ECG changes caused by *m*-AMSA alone are insufficient to induce disturbances in cardiac rhythm, but may increase ventricular vulnerability so that together with another precipitating factor such as hypokalemia, ischemia, or phenothiazines, serious ventricular arrhythmias may occur. We therefore recommend that the concomitant use of agents that may prolong the Q-T interval should be avoided during administration of *m*-AMSA and that the treating physician should be aware of the potential electrotoxic effect of the drug.

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Double Hemibody Irradiation in Chemotherapy-Resistant Multiple Myeloma[1]

P. J. Thomas,* A. Daban, and D. Bontoux[2]

Seven patients with high-mass multiple myeloma resistant to chemotherapy were treated by double hemibody irradiation (DHI). The total-body myeloma cell mass decreased from 38% to 80% for the five patients who received a complete treatment. Pain and performance status were improved. Hematological toxicity was severe, and one patient died after 3 months from marrow aplasia. Survival from 3 to > 19 months was obtained after the completion of DHI. According to our experiment, DHI can be considered as a treatment of last resort for resistant multiple myeloma. [Cancer Treat Rep 68:1173–1175, 1984]

The possibility of a prolonged remission in myelomatosis with total-body irradiation was proposed by Bergsagel (1). Different techniques of irradiation were introduced in the treatment of chemotherapy-resistant multiple myeloma (MM) (2–5). The first studies attempted to find an antalgic solution for patients beyond any therapeutic assistance. The efficacy of sequential radiotherapy on pain was demonstrated, and remissions of MM have been reported (3–5). Our experience with double hemibody irradiation (DHI) in chemotherapy-resistant MM confirmed these results.

METHODS

DHI was administered to seven patients followed at the Department of Rheumatology, Hôpital La Miletrie, Poitiers. All patients were severely ill before they were considered for DHI. All patients had high-mass tumors (6) and all tumors were secreting IgG (five) or IgA (two). Two patients had renal insufficiency associated with Bence Jones proteinuria (Patients 3 and 5). All patients failed to achieve remission with a therapeutic protocol including cyclophosphamide, melphalan, and prednisone given for at least 3 months. Two of these patients (Nos. 3 and 4) were not improved by a second course of chemotherapy consisting of doxorubicin, carmustine, and vincristine. Blood cell counts, M-component level, bone x-rays, and plasma volume were followed serially. Total-body myeloma cell mass (TBMC) and tumor mass regression were calculated periodically with the aid of an HP 97 calculator using the equations of Salmon and Wampler (7).

The radiotherapy was administered at 18 mV from a Saturne CGR Linear Accelerator. The effective distance was 2.06 m. A midplane dose of 8 Gy corrected for specific tissue absorption was delivered in a single treatment: 4 Gy through an anterior field and 4 Gy through a posterior field at a dose rate of 50 cGy/minute. Hemibody fields were divided at the umbilicus and a 5–6-week interval was allowed between each half. The eyes and lungs were shielded for the anterior field only. Except in one patient (No. 2), the inferior lower hemibody was irradiated first. Following each treatment session, vital signs were monitored regularly for at least 48 hours. Each session was accompanied by the systematic parenteral administration of a short-acting corticosteroid and an antiemetic. The two patients with renal insufficiency had systematic plasma exchange and urine alkalinization.

RESULTS

Lower hemibody irradiation was well-tolerated in all patients, and the hematological complications were mild. Clinical remissions were not observed in any of our seven patients. Nevertheless, the increase in tumor mass was stopped for three of the patients, and a decrease of 60% of the tumor was noted for another. The cell mass increased for three patients with myeloma. Two of the patients died before it was possible to perform the second part of the DHI. This shows that the patients were too advanced to be treated. Death was not related to irradiation but to tumor progression. Good hydration associated with urine alkalinization did not prevent a worsening of the renal condition for the two patients with renal failure.

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TABLE 1.—Effect of DHI on peripheral blood cell counts

Patient No.	Wbc count ($\times 10^3$ cells/mm^3)		Platelet count ($\times 10^3$ cells/mm^3)	
	Prior	Nadir	Prior	Nadir
1	3.2	2.5	172	43
2	2.9	1.7	202	129
3	2.0	0.9	103	18
4	3.1	1.0	105	31
5	2.5	1.2	109	30

TABLE 2.—Results of DHI on myeloma and survival after its completion

Patient No.	% maximum reduction of—		Performance status* variation	Survival (mos)
	TBMC	M component		
1	38	35	-2	11
2	60	55	-1	> 19
3	51	50	0	3
4	45	40	-3	19
5	80	75	-2	4

* Kyle index (*see* ref 8).

Upper hemibody irradiation was always badly tolerated. Fever, vomiting, and diarrhea were constant during the first 2 days. Anorexia and weakness lasted several weeks. The marrow function was strongly depressed (table 1). The wbc count nadirs occurred before the 10th day and the platelet count nadirs occurred between the 10th and the 30th day after the completion of DHI. Marrow recovery was achieved for all patients except one (No. 3). In this patient, marrow function improved initially, and aplasia appeared during the third month for no apparent reason. No pneumonitis, septicemia, or bleeding problems were observed. The renal condition of the two patients with insufficient kidney function worsened again, but plasma exchange returned the BUN to a preirradiation level after a few weeks. Hospitalization never exceeded 15 days after radiotherapy.

The five patients who received the complete treatment obtained a biological response, with a decrease of TBMC from 38% to 80% in a few months. The performance status, as scored by the performance index of Kyle et al (8), clearly improved in three patients, and bone pains decreased or disappeared for four patients. These results were maintained for several months. A survival from 3 to > 19 months was obtained after DHI (table 2).

DISCUSSION

The use of extensive irradiation for MM has been previously attempted (2–5). Because of the bad prognosis of their disease, patients unlikely to respond with conventional approaches are potential candidates for new approaches to treatment, including total-body or hemibody irradiation (4). DHI gave clear results on pain. The performance status was improved for several months in three of five patients who received the complete treatment. Even though TBMC reduction was observed in those patients, an improvement of survival is difficult to evaluate. The tumor reduction usually correlated with survival (4) and could indicate that these criteria are favorably influenced by DHI.

Chemotherapy usually results in a decrease of the TBMC and in an improvement of the disease. Unfortunately, relapse always occurs, sometimes early in the treatment. These patients with relapsing MM are usually resistant to alkylating agents (9). Increasing the doses or changing alkylating agents allowed a therapeutic response only rarely. Other chemotherapy modalities were proposed for the patients with MM but their efficacy was observed only when a partial response was noted with melphalan or with other alkylating agents (9). Patients with melphalan-resistant MM responded occasionally to another protocol (9,10), and DHI could represent second-line treatment for them.

DHI is a good palliative procedure for patients with severe bone pain secondary to advanced myeloma. Treatment toxicity was significant but tolerable. Furthermore, DHI presents a survival chance for patients whose expected life span is short.

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Susceptibility to Motion Sickness and the Development of Anticipatory Nausea and Vomiting in Cancer Patients Undergoing Chemotherapy[1,2]

Gary R. Morrow[3,4,*]

There are neural pathways between the vestibular system and an area of the brain in the fourth ventricle that appear to coordinate vomiting. Based on this and clinical observations, we theorized a relationship between a susceptibility to motion sickness and the intensity of nausea and vomiting from chemotherapy. A significant relationship ($P < 0.01$) between a susceptibility to motion sickness and anticipatory nausea/vomiting in cancer patients undergoing chemotherapy was found. Antimotion drugs such as scopolamine may have a greater antiemetic effect on chemotherapy patients susceptible to motion sickness than on those who are not susceptible. [Cancer Treat Rep 68:1177–1178, 1984]

Anticipatory nausea and vomiting are increasingly being seen as an important clinical problem in the management of chemotherapy-related side effects in cancer patients (1–6). Although there is some variation in incidence figures reported for anticipatory nausea and vomiting, it appears that at least one in four cancer patients experiences nausea/vomiting in anticipation of treatment once in a regimen of chemotherapy (2). During previous studies on the development of anticipatory nausea and vomiting (3) and a randomized clinical trial of its treatment (7), we noted a number of patients with anticipatory nausea and vomiting who reported chemotherapy-induced side effects that sounded similar to the experience of motion sickness. An involvement of the vestibular system in patients' response to the chemotherapy drugs is supported by neuropathways between the vestibular apparatus in man and a neural structure near the fourth ventricle called the vomiting center that appears to coordinate the expression of nausea and vomiting (8).

We hypothesized that a susceptibility to motion sickness would be involved in a patient's experience of anticipatory nausea and vomiting during chemotherapy treatments.

MATERIALS AND METHODS

Seven hundred consecutive ambulatory patients with histologically confirmed cancer were studied at their fourth treatment cycle. Patients were receiving chemotherapy from three geographically distinct hospitals of the University of Rochester Cancer Center. Approximately one-half of the patients studied had a diagnosis of breast cancer. Approximately one-quarter had a diagnosis of a hematopoietic malignancy. Patients were from 17 to 88 years of age, with a mean age of 56 years.

Patients reported previous susceptibility to motion sickness on a report questionnaire by answering the question "I have experienced motion sickness at some time (riding in cars, boats, planes)." They also reported their experience of nausea and vomiting in anticipation of any chemotherapy treatment on the same patient report questionnaire (7,9).

RESULTS

Overall, 608 of the 700 patients (87%) provided evaluable data. One hundred sixty-six of the 608 patients (27%) reported a susceptibility to motion sickness. Forty-

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eight of the 166 patients (29%) reporting susceptibility to motion sickness developed anticipatory nausea and vomiting. The incidence of anticipatory nausea and vomiting was only about one-half as high (17%) in patients not susceptible to motion sickness. One hundred twenty-two of the 608 patients (20%) reported anticipatory nausea and vomiting. Over 39% of the patients with anticipatory nausea and vomiting gave a history of susceptibility to motion sickness. One hundred eighteen of the 486 patients who did not report anticipatory side effects reported a susceptibility to motion sickness. A significant association ($\chi^2 = 11.17; P < 0.01$) between the report of a susceptibility to motion sickness and the experience of having anticipatory side effects was found.

DISCUSSION

These data support a view that the vestibular system (as assessed through a susceptibility to motion sickness) is involved in the development of anticipatory nausea and vomiting in chemotherapy patients. Previous studies in the dog have implicated the vestibular system in the expression of vomiting following the presentation of selected emetogenic agents (10). Data from the present study support a view that the vestibular system is also involved in the expression of nausea and vomiting in cancer patients undergoing chemotherapy.

Cancer patients with a susceptibility to motion sickness appear more prone to develop anticipatory nausea and vomiting during chemotherapy treatment than patients who are not susceptible. They may be a group for whom more intensive approaches of side effect management are needed. Cancer patients who report a susceptibility to motion sickness may be more responsive to antiemetic regimens that contain antimotion drugs such as scopolamine than patients who do not. Previous studies showing inconclusive results for scopolamine as an antiemetic for cancer patients (1) may have been confounded by inclusion of those patients who were not susceptible to motion sickness. Scopolamine or other antimotion medication may be differentially more effective as an antiemetic for patients with a susceptibility to motion sickness than for patients who are not susceptible.

Studies have shown the antiemetic effect of a behavioral approach called systematic desensitization in the management of anticipatory side effects. Combining this behavioral approach with an antimotion drug may result in more appropriate management of anticipatory nausea and vomiting in patients with a susceptibility to motion sickness than either approach alone may provide.

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Phase II Study of Vindesine, Cisplatin, and Hexamethylmelamine (VCH) in Small Cell Carcinoma of the Lung[1]

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Forty-two patients with small cell carcinoma of the lung were treated with cycles given every 28 days consisting of a combination of vindesine (3 mg/m² iv on Days 1 and 15), cisplatin (75 mg/m² iv on Day 1), and hexamethylmelamine (200 mg/m² orally on Days 8–22). Thirty-four patients were evaluable for response. Partial remission (PR) or complete remission (CR) was noted in seven (three CRs and four PRs) of nine previously untreated patients (77%) and in eight (one CR and seven PRs) of 25 patients treated previously with intensive chemotherapy (32%). This drug combination deserves consideration for inclusion in sequential combination chemotherapy regimens. [Cancer Treat Rep 68:1179–1181, 1984]

Despite improvements in the treatment of small cell carcinoma of the lung (SCCL) (1), the relapse rate is still distressingly high, and there is a clear need for more efficient therapeutic programs.

The current study was undertaken to evaluate the efficacy of a combination of vindesine, cisplatin, and hexamethylmelamine (VCH) in previously untreated patients with SCCL and in patients with refractory disease previously treated with the most active agents in SCCL, ie, vincristine, etoposide, methotrexate, cyclophosphamide, doxorubicin, and lomustine.

MATERIALS AND METHODS

Nine previously untreated patients and 25 patients who progressed while receiving primary treatment were included in the study. All patients had histologically confined small cell carcinoma according to World Health Organization (WHO) criteria. Informed consent was given by all patients.

Treatment with VCH was not initiated until hematologic restitution after prior therapy had occurred. A wbc count of $\geq 4 \times 10^9$/L and a platelet count of $\geq 100 \times 10^9$/L were required unless there was biopsy-proven bone marrow involvement. Normal renal function estimated by ^{51}Cr-EDTA clearance was required. Criteria for inclusion also included measurable or evaluable disease and an expected survival of at least 4 weeks.

Clinical examination was performed before the start of therapy and every 2 weeks thereafter. Cbcs were done every 2 weeks. Serum electrolytes, creatinine, alkaline phosphatase, LDH, bilirubin, prothrombin time, ^{51}Cr-EDTA clearance, and chest roentgenograms were performed every 4 weeks. Performance status was scored according to the WHO recommendations (2).

Chemotherapy given on a 28-day cycle consisted of vindesine at a dose of 3 mg/m² iv on Days 1 and 15 (every 7 days for the first 28 days), cisplatin at a dose of 75 mg/m² iv on Day 1, and hexamethylmelamine at a dose of 200 mg/m² orally on Days 8–22. Immediately before infusion of cisplatin the patients were hydrated with 1500 ml of isotonic NaCl/glucose for 2 hours and after cisplatin they received 2000 ml of isotonic NaCl/glucose for 2 hours. The chemotherapy dose was modified according to side effects. However, in cases of proven bone marrow involvement, no modifications were made.

The previously untreated patients received VCH for a maximum of 56 days and treatment was thereafter continued with "conventional" combination chemotherapy as described elsewhere (3). The patients who had previously received chemotherapy were treated until disease progression. Standard response criteria according to WHO were employed (2).

RESULTS

Eight of 42 patients entered in the study were considered inevaluable for response: four because of early

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death (< 2 weeks after start of VCH, without signs of toxicity), three because of severe abdominal pain due to hexamethylmelamine, necessitating discontinuation of the drug already in the first cycle, and one because of inevaluable disease. Characteristics of the nine previously untreated patients and the 25 previously treated patients are shown in table 1. The overall complete response (CR) and partial response (PR) rate was 77% (40%–95% confidence limits) for previously untreated patients, with three patients (33%) achieving CR and four (44%) achieving PR. Two of the patients achieving CR were still without evidence of disease after 18 months of additional treatment with combination chemotherapy consisting of vincristine, methotrexate, cyclophosphamide, etoposide, doxorubicin, and lomustine, with the restaging procedures including bronchoscopy, bilateral bone marrow biopsies, and peritonoscopy with liver biopsy. The overall CR and PR rate for 25 previously treated patients was 32% (15%–54%, 95% confidence limits), with one patient (4%) achieving CR and seven (28%) achieving PR. The duration of response for the patient with CR was 54 days, with a median response of 83 days (range, 47–191) for patients with PR. Five of seven patients responding to this treatment (CR + PR) had limited disease and two had extensive disease. None of these patients had previously achieved CR. Seventeen of the 25 patients (68%) who had initially responded were refractory to VCH. Two of six patients (33%) with primary resistance responded to VCH, compared to six of 19 patients (33%) who had initial response to the first-line therapy. The median survival of the previously treated patients was 117 days (range, 51–281) after starting VCH. In 37 patients evaluable for toxic effects, myelosuppression and nausea and vomiting were the most prominent problems. Dose reduction of myelosuppressive agents, usually to 50%–67% of the scheduled dose, was required in 11 patients. All of these patients received more than one course of chemotherapy. Toxic effects are shown in table 2. There were no drug-related deaths.

TABLE 1.—Characteristics of patients treated with VCH*

Characteristic	Previously treated patients	Previously untreated patients
Evaluable for—		
Response	25	9
Toxic effects	28	9
Median age in yrs (range)	64 (45–71)	58 (38–69)
Sex		
Males	24	4
Females	9	5
Limited disease	20	7
Extensive disease	13	2
Performance status		
0–1	10	5
2	16	2
3	7	2
Chest irradiation	3	1
Prior chemotherapy	33	0
Prior CNS irradiation	1	0

* Unless otherwise specified, values = No. of patients.

DISCUSSION

The response rate with cisplatin as a single agent in SCCL varies from 6% to 30%, presumably reflecting the varying dose of cisplatin and the intensity of antineoplastic therapy given to patients before receiving cisplatin (4,5). The response rates for vindesine and hexamethylmelamine are 27% and 26%–43%, respectively (6–8). The latter was obtained in previously untreated patients. It is noteworthy that vindesine is also active in patients who have previously received vincristine (6).

Contrary to the usual low response rate in patients with relapsed or progressive SCCL, VCH produced an overall response rate of 32% lasting ≥ 4 months in three patients. A response rate of 77% in previously untreated patients is comparable to results in other trials including untreated patients (9). It is not possible to conclude anything about the influence of VCH treatment on median survival in these patients because the subsequent treatment included combinations with active agents. The present study suggests, however, that VCH represents a therapeutic option for patients relapsing with SCCL and that VCH is not cross-resistant to the primary combination of the six drugs mentioned previously.

The use of non-cross-resistant drug regimens is one way of widening the spectrum of cytotoxic activity against heterogeneous cell populations as seen in SCCL, and a recent study has also provided theoretical support for such a treatment strategy (10). Poplin et al (11) and Joss et al (12) have evaluated the response to the second, supposedly non-cross-resistant combination after failure with the primary combination, and found response rates of 31% and 8%, respectively.

VCH is now included in an ongoing phase III trial in an attempt to elucidate whether the overall treatment results will be improved by incorporation of this combination with other non-cross-resistant drug regimens in a sequential fashion.

TABLE 2.—Toxicity of VCH

Toxic effect	No. of patients
Wbc count < 2.0×10^9/L	11
Septicemia	1
Pneumonia	6
Platelet count < 100×10^9/L	8
Nausea and vomiting	28
Severe abdominal pain	3
Paresthesias/muscular pain/weakness	6

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Phase II Study of PALA, Amsacrine, Teniposide, and Zinostatin in Small Cell Lung Carcinoma (EST 2579)[1,2]

Richard H. Creech,* David Tritchler, David S. Ettinger, John A. Ferraro, John C. Ruckdeschel, Steven E. Vogl, and Judith Woll[2]

Eighty-two patients with small cell lung carcinoma refractory to standard chemotherapy were entered in this phase II randomized study of PALA, amsacrine, teniposide, and zinostatin. Of the 66 evaluable patients, one partial response occurred among 17 patients treated with teniposide and no responses occurred with the other drugs. Two patients each treated with amsacrine and teniposide experienced life-threatening hematologic toxic effects and one patient treated with zinostatin died of thrombocytopenic pulmonary hemorrhage. The overall median patient survival was 9.6 weeks. Weight loss ≥ 5% prior to therapy, extensive disease, and a nonambulatory status were associated with poor survival. [Cancer Treat Rep 68:1183–1184, 1984]

In this phase II study of small cell lung carcinoma refractory to standard chemotherapy, patients were randomized to receive one of four chemotherapeutic agents that had not previously received adequate clinical trial: PALA (*N*-phosphonacetyl-L-aspartate), a water-soluble transition-state inhibitor of aspartate transcarbamylase, an important enzyme in de novo pyrimidine biosynthesis; the acridine derivative amsacrine; the podophyllin derivative teniposide; and zinostatin, a protein antibiotic isolated from a culture filtrate of *Streptomyces carzinostaticus* var F41.

PATIENTS AND METHODS

Patients were stratified by Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–1 (ambulatory) versus PS 2 (nonambulatory) and by disease extent (limited vs extensive).

To be eligible for the study, patients had to have histologically confirmed small cell bronchogenic carcinoma, measurable or evaluable recurrent or metastatic disease, progression after treatment with standard chemotherapeutic agents, PS 0–2, wbc count ≥ 4000/mm^3, platelet count ≥ 100,000/mm^3, BUN ≤ 20 mg/100 ml, creatinine ≤ 1.5 mg/100 ml, bilirubin ≤ 2.0 mg/100 ml, no prior therapy with any of the study drugs, no chemotherapy for 3 weeks (6 weeks for nitrosoureas), and no brain metastases. All patients had to give informed written consent prior to participation in this study.

Patients randomized to PALA received 5 g/m^2 iv over 15–30 minutes every 14 days. Amsacrine was given at 120 mg/m^2 iv in 5% dextrose in water every 21 days. Teniposide was administered at 100 mg/m^2 iv in 250 ml of 5% dextrose in water over one-half hour every 7 days. Zinostatin was given at 2 mg/m^2 iv in 150 ml of 5% dextrose in water over one-half hour daily for 5 days at 28-

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day intervals. The zinostatin patients, who were premedicated with acetaminophen and diphenhydramine hydrochloride, had their intravenous tubing and bag protected from light. Each drug was given for at least 28 days or until there was clinical evidence of progressive disease. At the time of progression, patients were not eligible for treatment with the other study drugs.

Objective tumor response was classified according to standard ECOG response criteria and treatment complications were reported according to standard ECOG criteria by type, cause, and degree (none, mild, moderate, severe, life-threatening, or lethal) (1).

RESULTS

Eighty-two patients were entered in this study between May 1980 and March 1982. Seventeen of 21, 16 of 21, 17 of 19, and 16 of 21 patients admitted respectively to the PALA, amsacrine, teniposide, and zinostatin arms were analyzed. Sixteen patients were not evaluable because of five cancellations and 11 cases of ineligibility. These 16 patients were not analyzed because of unacceptable pretherapy laboratory values (five), death prior to initiation of therapy (two), brain metastases (one), PS > 2 (two), non-small cell histology (two), chemotherapy within 3 weeks of on-study date (two), or a lack of evaluable/measurable disease (two).

The 66 evaluable patients had previously been extensively treated with multiple drug regimens. Eleven patients had received one regimen, 42 had received two regimens, 11 had received three regimens, and two had received four regimens.

There was only one partial response among the 66 evaluable patients. In this male patient, one of 17 patients treated with teniposide, there was a partial regression of a hilar node on Day 22 for 17 weeks, with a survival of 59+ weeks.

Severe, life-threatening, or lethal toxic effects occurred in two patients treated with PALA, six treated with amsacrine, four treated with teniposide, and three treated with zinostatin. PALA was associated with one severe rash and one instance of severe mucositis. Amsacrine was associated with three cases of severe neutropenia and one case of severe nausea and vomiting. One patient treated with amasacrine had life-threatening neutropenia and thrombocytopenia and another had life-threatening neutropenia. Teniposide caused one instance of severe neutropenia and another of severe neutropenia and thrombocytopenia. This drug was also associated with life-threatening neutropenia and thrombocytopenia in another patient. Zinostatin was associated with severe thrombocytopenia in two patients and a lethal thrombocytopenia-associated pulmonary hemorrhage in another.

The median survival in weeks for the four treatment groups was: PALA—10.1, amsacrine—11.4, teniposide—10.3, and zinostatin—6.4. The overall median survival was 9.6 weeks. Only two patients survive.

An analysis of the survival data was conducted using the proportional hazards models proposed by Cox (2-4). The variables evaluated included treatment, age, sex, disease extent, initial PS, prior weight loss, prior radiotherapy, and previous surgery. The following characteristics were correlated with poor survival: increasing weight loss during the 6 months prior to initiation of protocol therapy ($P = 0.0003$); extensive disease ($P = 0.01$); and poorer PS ($P = 0.03$).

DISCUSSION

In this phase II ECOG study of small cell lung carcinoma, one patient had a partial response of a hilar node with teniposide, while 16 had no response. Eighteen patients treated with PALA, 16 treated with amsacrine, and 13 treated with zinostatin had no response. These data could indicate that none of these drugs are effective in small cell lung cancer or that these extensively previously treated patients may not be appropriate for detecting possibly effective drugs. Since response rates decrease with successive chemotherapy regimens and with a worsening PS, it is recommended that future phase II studies of small cell lung cancer be limited to ambulatory patients after their first chemotherapy failure.

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Experience With the Colony-Forming (Stem Cell) Assay of In Vitro Chemosensitivity in the Management of Patients With Advanced Malignant Melanoma[1,2]

Rona M. MacKie,* J. M. Gaukroger, Lesley Wilson, and Jean Gold[3]

Twenty-two patients with advanced malignant melanoma had tumor nodules biopsied and tumor cell colonies established in vitro in the colony-forming assay in the presence of vindesine. The patients then received iv vindesine at a dose of 3 mg/m²/week for 6 weeks. In four patients, in vitro death of cells exposed to vindesine in vitro correlated with clinical response to therapy. In the remaining 18 patients, lack of both in vitro and clinical response was noted. [Cancer Treat Rep 68:1185–1188, 1984]

While the concept of in vitro chemosensitivity testing of tumor cells from individual patients is attractive, there are certain problems. The theory is based on the premise that the cells which survive and grow to form colonies in soft agar culture are those which are the "growth fraction" of the tumor in vivo. The recent work by Thomson and Meyskens (1) on the self-renewing capacity of cells which establish colonies in this system is important evidence that this theory is correct.

In 1977, an in vitro assay (stem cell or colony-forming) was introduced by Hamburger and Salmon (2), Meyskens et al (3), and Salmon et al (4). This assay was based on the fact that only malignant cells will grow in soft agar cultures and therefore overgrowth by benign fibroblasts or other cell types does not occur.

The two drugs which at present are considered to be of greatest value in advanced melanoma are dacarbazine (5) and vindesine (6). Both, however, have response rates of only 20%–26%, and the duration of response is relatively short.

Therefore, it would clearly be of value to have an in vitro assay capable of identifying those 75% of patients for whom chemotherapy is of no value, saving needless toxicity and unpleasant side effects. Biopsy samples have therefore been taken from 22 patients with advanced malignant melanoma prior to vindesine therapy and the clinical response to this drug was compared to the in vitro growth characteristics.

PATIENTS AND METHODS

Patients

Twenty-two men and women had biopsies of accessible tumor deposits. Details of age, tumor distribution, and clinical course after chemotherapy are shown in table 1. All patients had vindesine at a dose of 3 mg/m² given iv by fast drip once a week for 6 weeks. The vindesine infusion was delayed if the wbc count fell to < 3000/mm³.

Methods

Preparation of biopsy specimen.—Tumor tissue was mechanically dissociated into a single-cell suspension in RPMI-1640 medium containing antibiotics. This crude suspension was filtered through sterile gauze and centrifuged and the cells were resuspended in fresh medium. Cell numbers were then determined, and viability was estimated by trypan blue dye exclusion (0.2% wt/vol).

Salmon assay technique.—Petri dishes (35 mm²) were prepared essentially as described by Soehnlen et al (7), with a 0.5% agar feeder layer and an upper 0.3% agar cell layer (both containing enriched medium). The assay was carried out in triplicate at final vindesine concentrations of 5×10^{-9} and 5×10^{-6} M. Vindesine was added to each cell suspension immediately prior to addition of the agar, and 1-ml aliquots of this mixture containing 5 ×

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10^{-5} cells were distributed to each dish. Control dishes contained no vindesine. The dishes were examined after 24 hours and any containing clumps of cells rather than single-cell suspensions were discarded. The incubation vessels were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Concentrations of vindesine similar to those used by Soehnlen et al (7) and Alberts et al (8) were assessed by incorporation of the drug in the agar to achieve a continuous contact with the cells. Colonies (> 32 cells) were counted 14-21 days after inoculation at maximum colony formation. Colony formation inhibition in dishes containing vindesine by comparison with colony formation in dishes with no vindesine was calculated and expressed as a percentage (> 70% = positive result).

RESULTS (Table 1)

The clinical response rate to iv vindesine was 18% (four responses among 22 patients). In all four patients, the in vitro results obtained by the Salmon technique showed significant cell kill (≤ 53% inhibition of colony formation), and therefore appeared to be an accurate predictor of clinical response. In the remaining 18 patients, inhibition of colony formation was < 70% or showed a "mixed" response, which implies sensitivity only at the higher concentration of 5×10^{-6} M vindesine. All of these patients showed continuing steady growth of measurable tumor during and after vindesine therapy. Once again, total correlation of in vitro response with clinical behavior was achieved. Biopsies of three patients in this latter group exhibited enhanced tumor growth on exposure to vindesine and clinical deterioration correlated with this in vitro observation.

Table 2 shows detailed results for six of our patients, with plating efficiencies ranging from 0.007% to 0.074%, similar to those reported by other investigators. Relative colony counts (RCC) for Patients 10, 14, 21, and 22, all of whom showed positive in vitro/in vivo sensitivity correlation, are shown. Two samples (from Patients 4 and 16) show a partial response and Patient 15 shows a resistant in vitro pattern.

TABLE 1.—Patient characteristics and in vitro and in vivo responses to vindesine

Patient No.	Age (yrs)	Sex	Site of primary	Site of metastases*	In vitro predictive test	Clinical progress
1	69	F	Cheek	Neck area	Resistant	Progression
2	62	F	Neck	Local recurrence	Resistant	Progression
3	60	M	Back	Soft tissue and lungs	Resistant	Progression
4	37	M	Scapular area	Multiple soft tissue	Resistant	Progression
5	30	F	Thigh	Multiple soft tissue and brain	Resistant	Progression
6†	82	F	Ankle	Local recurrence and lymph node	Resistant	Progression
7	46	F	Leg	Chest and soft tissue	Resistant	Progression
8	42	M	Leg	Massive local recurrence and liver	Resistant	Progression
9†	31	F	Neck	Multiple soft tissue and liver	Resistant	Progression
10	74	F	Leg	Multiple local recurrence	Responsive	Shrinkage
11†	42	M	Arm	Multiple subcutaneous recurrence	Resistant	Progression
12	66	M	Arm	Multiple soft tissue	Resistant	Progression
13	42	F	Hand	Soft tissue and lungs	Responsive	Shrinkage
14	71	M	Calf	Local recurrence	Responsive	Shrinkage
15	21	F	Arm	Multiple soft tissue	Resistant	Progression
16	74	F	Leg	Groin	Resistant	Progression
17	52	F	Face	Extensive local recurrence	Resistant	Progression
18	68	M	Mucosal	Subcutaneous lesions, back	Resistant	Progression
19	53	F	Leg	Soft tissue and liver	Resistant	Progressive
20	61	F	Leg	Soft tissue	Resistant	Progressive
21	52	F	Face	Soft tissue	Sensitive	Partial response
22	69	M	Leg	Pulmonary	Sensitive	Partial response

* High proportion of patients with subcutaneous metastases is explained by the fact that these are accessible to biopsy and subsequent assay.

†Biopsies showed apparent *enhanced* tumor cell growth in contact with vindesine in vitro.

DISCUSSION

This study indicates that for a specific tumor type (melanoma) and a specific cytotoxic drug (vindesine), the Salmon colony-forming assay is a useful and accurate in vitro predictor of in vivo behavior. We are confident that it can be used to screen patients for whom vindesine therapy would be ineffective and thus prevent needless drug-induced toxicity.

The observation of apparent enhanced growth of three biopsy samples in the presence of vindesine is a worrisome observation and is perhaps in vitro evidence of the recent hypothesis (9), suggesting that chemotherapy may, in certain situations, kill off less aggressive clones and thus allow outgrowth of those cells with the greatest metastatic potential which will eventually kill the patient. This suggestion clearly requires further study and confirmation.

Patients 10 and 22, who responded in vivo, have sensitivities to 5×10^{-6} M vindesine in vitro of 20.4% and 39.3%, respectively, which agree with the criteria of Meyskens et al (3).

Two other patients (Nos. 14 and 21), who exhibited a response in vivo, had in vitro sensitivities of 46.8% and 47.2%, respectively, exceeding the criteria of Meyskens by approximately 9%. This compares with sensitivities of 54.5% and 54.8% to 5×10^{-6} M vindesine for two patients (Nos. 4 and 16) in whom no in vivo response was apparent.

If the sensitivities of Patients 4 and 16 (responsive in vivo) at 10^{-6} and 10^{-9} M vindesine are compared to that of Patient 14 (unresponsive in vivo), there is an apparent difference in the sensitivities at the two concentrations.

For the nonresponding patients there is no major increase in sensitivity when the concentration of vindesine is increased from 10^{-9} to 10^{-6} M (differences of 7.5% and 9.4%, respectively), whereas for Patient 14 who responded in vivo there is a decrease in the number of tumor colony-forming units to almost one-half (a difference of 40.9%). This indicates that a result obtained at any single-drug concentration would not necessarily reveal sensitivity and/or resistance unless the colony formation was < 40% of the control; for a reliable indication, two-drug concentrations should be used for each cytotoxic agent.

Our results were obtained using a regimen where cells were exposed continuously to the cytotoxic agent (vindesine). Other investigators (4) have done cytotoxicity tests employing a 1-hour exposure to the drug and related this to the plasma concentration-time product to assess sensitivity. Recently, Rupniak et al (10) have reported that this is inappropriate for some cell-cycle phase-specific agents such as hydroxyurea and that longer exposure times are required to evaluate cytotoxicity. Although these investigators did not find as great a time-dependent difference for vinblastine action on ovarian carcinoma cells, they used the Courtney assay procedure. Al-

TABLE 2.—Details of colony growth characteristics

Patient No.	Relative plating efficiency*	Colony growth: Control Mean	Control SD	Vindesine (5×10^{-9} M) Mean	Vindesine (5×10^{-9} M) SD	Vindesine (5×10^{-9} M) RCC†	Vindesine (5×10^{-6} M) Mean	Vindesine (5×10^{-6} M) SD	Vindesine (5×10^{-6} M) RCC†	Response in vivo
4	0.02	100.7	17.7	110	8.5	109	89	18.7	88.4	No
10	0.014	54.0	12.0	—	—	—	11	1.0	20.4	Yes
14	0.016	79.0	8.0	68.5	1.5	86	37	—	46.8	Yes
16	0.024	121.0	16.4	75.3	5.2	62	66	7.0	54.8	No
21	0.007	36.0	5.7	—	—	—	17	0	47.2	Yes
22	0.072	361	29.2	—	—	—	141.7	12.5	39.3	Yes

* Relative plating efficiency = $\frac{\text{No. of colonies/dish}}{\text{No. of cells plated/dish}} \times 100\%$.

†RCC = $\frac{\text{No. of colonies/drug dish}}{\text{No. of colonies/control dish}} \times 100\%$.

berts et al (8) reported a similar action for vinblastine and vindesine in melanoma.

Despite these problems of continuous and limited contact of drug and cells, our results show that for vindesine, continuous contact in the double-layer agar technique results in colony survival that gives a positive indication of the in vivo response to this agent. The colony-forming or stem cell assay is thus a useful guide to chemotherapy in melanoma.

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Murine Leukemia L1210 Cell Lines With Different Patterns of Resistance to Platinum Coordination Complexes[1,2]

Alan Eastman* and Sharon Illenye[3,4]

A murine leukemia L1210 cell line has been developed that exhibits 30-fold resistance to 1,2-diaminocyclohexaneplatinum(II) analogs but retains sensitivity to cisplatin. This contrasts existing cell lines which exhibit the opposite pattern of resistance. Both cell lines retain their sensitivity to melphalan. [Cancer Treat Rep 68:1189–1190, 1984].

The development of resistance in tumors is frequently considered to be a major limitation in the use of cancer chemotherapeutic agents. It was originally thought, at least for alkylating agents, that resistance to one drug meant cross-resistance to all others (1). Subsequent experiments have demonstrated that many cell lines exhibit variable patterns of cross-resistance (2–4). In this regard, various murine leukemia L1210 cell lines have been developed which are resistant to cisplatin (*cis*-DDP) (5), a drug whose action is thought to depend on its interaction with DNA (6). Previous investigators have reported that L1210 cell lines with acquired resistance to melphalan were cross-resistant to *cis*-DDP (2,7), whereas L1210 cells, whose resistance was initially developed against *cis*-DDP, retained their sensitivity toward melphalan (4,7). It also appears that L1210 cells can exhibit differential sensitivity toward specific platinum analogs. Therefore, one *cis*-DDP-resistant cell line, L1210/DDP, is still sensitive to diaminocyclohexane analogs (5). In part because of this observation, carboxyphthalato-1,2-diaminocyclohexaneplatinum(II) (DACH-Pt-CP) is now entering clinical trials. It seems likely that resistance could also be developed to this platinum analog. The development of such a resistant cell line is presented here.

MATERIALS AND METHODS

The drugs were obtained from the following sources: *cis*-DDP (platinol) and DACH-Pt-CP (JM-82), Bristol Laboratories, Syracuse, NY; *trans*-DDP, Alfa Ventron, Danvers, MA; melphalan (Alkeran), Burroughs Wellcome Co, Research Triangle Park, NC; and DACH-Pt-SO_4 and DACH-Pt-ascorbate were synthesized and donated by Dr. David Brown, Department of Chemistry, University of Vermont. Melphalan and *cis*-DDP were dissolved according to the manufacturer's recommendations. DACH-Pt-CP was dissolved in 2% sodium bicarbonate. Other drugs were dissolved in water. Solutions of DACH-Pt-CP and *cis*-DDP retained activity for at least 1 month. All other drugs were used immediately upon dissolving. All culture media and supplies were obtained from Gibco, Grand Island, NY.

Detailed procedures for maintenance of L1210 cells and growth inhibition studies have been reported previously (4). The results are expressed as ID50, defined as the drug concentration that caused 50% inhibition of cell growth relative to untreated control cells as measured after a 3-day growth period in the presence or absence of drug.

RESULTS AND DISCUSSION

In initial experiments L1210/0 cells were incubated in a range of *cis*-DDP concentrations from 0.1 to 1 μg/ml. When cell growth neared saturation the cells were diluted 1:5 into the same concentration of drug. At the higher concentrations it was possible to subculture cells several times but the accumulated damage was eventually lethal. It was found that a dose about twice the ID50 was optimal for developing resistance. At lower doses re-

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[4]We are grateful to the late Dr. David Brown, University of Vermont, who developed our interest in these platinum analogs and provided samples for this research. We also thank Bristol Laboratories for the gift of DACH-Pt-CP.

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TABLE 1.—Effects of various agents on the growth of sensitive and resistant L1210 cells

Drug treatment	ID50 (μg/ml)		
	L1210/0	L1210/DDP	L1210/DACH
cis-DDP	0.12	7.12 (59)*	0.37 (3.1)
trans-DDP	11.30	38.00 (3.4)	19.00 (1.7)
DACH-Pt-carboxyphthalate	0.45	0.65 (1.4)	13.00 (29)
DACH-Pt-SO_4	0.33	1.17 (3.5)	10.30 (31)
DACH-Pt-ascorbate	1.10	2.88 (2.6)	44.00 (40)
Melphalan	0.33	2.23 (6.8)	0.79 (2.4)

* Values in parentheses represent -fold resistance.

sistance did not appear to develop to any significant extent. The concentration of drug was increased slowly as the cells appeared to better tolerate the applied dose.

L1210/0 cells have been grown in increasing concentrations of both *cis*-DDP and DACH-Pt-CP for 18 months. The rate that resistance was developed was approximately the same with both drugs. A slight increase in resistance was observed during the first 3 months followed by a step up to 20-fold resistance at 6 months. Thereafter, resistance has increased slowly. The cells are presently maintained in either 10 μg/ml of *cis*-DDP or 8 μg/ml of DACH-Pt-CP. Resistance was not reduced by inhibition for 2 months in the absence of either drug. We have also been attempting for 4 years to increase the resistance of the original L1210/DDP cells, although with limited success. Our best cell line to date has only 100-fold resistance to *cis*-DDP. It appears that some limit may exist as to the extent of attainable resistance.

A comparison was made of the sensitivity of the three cell lines, L1210/0, L1210/DDP, and L1210/DACH, to a variety of platinum analogs as well as melphalan (table 1). L1210/DDP exhibited resistance only to *cis*-DDP, while L1210/DACH was resistant to three DACH-Pt analogs but was still sensitive to *cis*-DDP. Only a relatively small degree of resistance to melphalan was observed in either cell line.

The DACH-Pt analogs differ only with respect to their leaving groups. The dichloride was not used because of its insolubility. The ascorbate is, however, an extremely soluble complex that appears to have considerable potential in cancer chemotherapy, particularly as it exhibits little, if any, renal toxicity (8). The fact that L1210/DACH cells are equally resistant to these DACH-Pt analogs suggests that they have the same mechanism of action. However, the mechanism of action may be different for *cis*-DDP. The first possibility to be explored is that reduced uptake causes the observed resistance. This possibility has now been ruled out for *cis*-DDP resistance in L1210/DDP cells (unpublished observations), although the possibility that uptake contributes to resistance to DACH-Pt has still to be explored.

The present study has demonstrated that resistance can develop to DACH-Pt as well as to *cis*-DDP and that the rates of development of resistance are the same for both drugs. However, the cell lines exhibited no cross-resistance with the alternate drug. The development of these cell lines will provide a useful model for analysis of the mechanisms of action and mechanisms of resistance to platinum coordination complexes.

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Phase I Study of the Combination of Iv Mitomycin and Oral Metronidazole[1]

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It has been suggested that hypoxic cell radiosensitizers will enhance the cytotoxic activity of chemotherapeutic agents in solid tumors as shown in animal experimental models (1-3).

We have evaluated the combination of mitomycin, an antitumor antibiotic with activity against a wide range of solid tumors which probably acts as an alkylating agent, together with metronidazole, a 5-nitroimidazole compound which was found to be effective clinically as a radiosensitizer (4) and to potentiate the activity of alkylating agents in vitro and in animal tumor models (3). We chose metronidazole because of its availability and relative lack of toxicity.

We combined iv mitomycin, using conventional doses (20 mg/m^2), and increasing doses of metronidazole (tables 1 and 2), which was administered orally as a flavored syrup 1 hour before mitomycin. This schedule was chosen because maximum serum levels are reached about 1 hour after injection of a small volume of metronidazole (5).

Sixteen patients with solid tumors (table 1) that progressed while receiving prior chemotherapy received the combination of mitomycin and metronidazole which was repeated every 6 weeks and delayed if the platelet count was < 100,000/mm^3 or the wbc count was < 3500/mm^3.

One partial response (6%) lasting 4 months was seen in a patient with adenocarcinoma of unknown origin, whose lung and liver metastases decreased > 50% in size. Three patients with stomach, colon, and breast carcinomas had responses of < 50% (minor response). Four patients had stable disease and eight showed progression of disease.

The dose-limiting toxic effect in our study was nausea and vomiting. Hematological toxicity was relatively mild (tables 1 and 2). Only one case of transient peripheral neuropathy was observed which did not recur when the patient received further increased doses of metronidazole. The maximum tolerated dose of oral metronidazole, when given concomitantly with 20 mg/m^2 of mitomycin every 6 weeks, was 7 g/m^2. At doses greater than this, vomiting prevented absorption of the metronidazole. This is similar to the maximum tolerated dose of metronidazole when given in conjunction with radiotherapy (4).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	16
Males/females	6/10
Median age in yrs (range): 54 (35-71)	
No. of courses evaluated: 34	
Median courses/patient (range): 2 (1-7)*	
Primary tumor†	
Colon	6
Gastric	4
Pancreas	1
Breast	4
Unknown primary	2
Response (14 evaluable patients)‡	
Partial	1 (4)
Minor	3 (2, 4, 12)
Stable disease	4 (2, 4, 7, 8)
Disease progression	6
Toxic effects	
Wbc count < 3500/mm^3	2
Platelet count < 100,000/mm^3	3
Transient peripheral neuropathy	1
Extravasation	1
Gastrointestinal	§

*For 4 patients, 20-mg/m^2 dose of mitomycin was reduced by up to 30% because of previous myelotoxic therapy.
†1 patient with double primary: gastric and colon.
‡Duration of responses (mos) shown in parentheses.
§*See* table 2.

TABLE 2.—Gastrointestinal toxicity

Dose of metronidazale (g/m^2)	No. of courses given	No. of courses (%) with severe nausea and vomiting
2-3	3	0 (0)
4	4	1 (25)
5	3	0 (0)
6	13	4 (31)
7	8	3 (37)
8	3	3 (100)

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The antitumor effect of mitomycin with metronidazole was minimal in our study. This may be due to the patient population, all of whom had advanced solid tumors refractory to previous therapy. A study of iv metronidazole and mitomycin is planned. The parenteral route may circumvent the gastrointestinal toxicity and permit administration of higher doses of metronidazole.

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Phase II Study of Diaziquone in Patients With Advanced Carcinoma of the Lung[1]

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The aziridinylbenzoquinones constitute a class of compounds possessing physical and chemical properties conducive to penetration of the central nervous system (1). A comparison of 31 aziridinylbenzoquinones in two intracerebrally and three intraperitoneally implanted murine tumor systems demonstrated diaziquone (AZQ) to be the superior analog (2). A phase I study of AZQ by our group showed myelosuppression to be the dose-limiting toxic effect (3).

PATIENTS AND METHODS

All patients were required to have histologic confirmation of metastatic lung cancer; measurable or evaluable disease; normal hematologic, renal, and hepatic parameters; age < 75 years; performance score (Eastern Cooperative Oncology Group [ECOG] scale) of 0-3; and no previous pelvic radiation therapy > 2500 rads.

Forty patients were entered in the study and all were evaluable for response. Patient characteristics are shown in table 1. Most of the previously treated patients had received cyclophosphamide, doxorubicin, and cisplatin; 5-FU, doxorubicin, and mitomycin; or a dibromodulcitol-based regimen. Patients received AZQ at a dose of 22.5 mg/m^2 iv over a 10-minute period once every 4-5 weeks.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	40
Males/females	25/15
Prior chemotherapy (yes/no)	29/11*
Median age (yrs): 63	
ECOG performance score	
0-1	14
2-3	26
Histologic type	
Epidermoid carcinoma	7
Adenocarcinoma	22
Large cell carcinoma	7
Small cell carcinoma	4
Hematologic toxicity	
Leukopenia (cells/mm^3)	
3000-3999	9
2000-2999	5
1000-1999	3
< 1000	1
Thrombocytopenia (cells/mm^3)	
50,000-100,000	5
25,000-49,999	6
< 25,000	1

* 4 patients with prior radiation therapy only.

RESULTS

Hematologic suppression was the most commonly encountered toxic effect. The incidence of thrombocytopenia and leukopenia was less in previously untreated patients. The median number of doses given was one per patient (range, one to six). The one patient receiving six courses of AZQ (dose range, 22.5-27.0 mg/m^2) had significant myelosuppression (leukopenia, 1600 cells/mm^3; thrombocytopenia, 38,000 cells/mm^3) following the highest dose. Minimal nausea and vomiting was noted in five patients.

There were no objective responses. The median time to disease progression was 29 days (range, 11-219) and the median survival time was 112 days (range, 11-503).

DISCUSSION

Hematologic toxicity and lack of therapeutic response noted in our study are similar to those reported by others in the evaluation of AZQ in patients with lung cancer (4). Because of the high lipid solubility of AZQ, it was hoped that this agent might prove useful in treating cerebral metastases. This was not the case, since four patients developed either new cerebral metastases or had an increase in size of previous metastases while receiving AZQ. AZQ does not appear to have any substantial activity in patients with advanced lung cancer.

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Phase II Trial of Etoposide in Advanced Prostate Cancer[1,2]

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In view of the activity of etoposide in a variety of human tumors and data suggesting enhanced efficacy when a continuous-infusion schedule is employed, we evaluated continuous-infusion etoposide in patients with advanced prostate cancer (1-3).

METHODS

Twenty-one men with measurable or evaluable metastatic prostate cancer and symptomatic progressive disease following therapy with diethylstilbestrol (> 3 mg/day) or bilateral orchiectomy were treated. Patients whose only evidence of disease was an abnormal bone scan or blastic bone lesions were not eligible for study. Twenty patients had increased serum acid phosphatase values, 21 had bone metastases, and 13 had evidence of soft tissue infiltration. Soft tissue disease was evaluable only by pelvic computerized tomographic scan in eight of the 13 patients. All patients had a performance status of ⩽ 3 and all had adequate renal function (creatinine < 2.0 mg/dl), hepatic function (bilirubin < 2.0 mg/dl), and bone marrow reserve (wbc count > 3500/mm^3 and platelet count > 100,000/mm^3). No patient had received prior treatment with etoposide. All patients gave informed consent.

Etoposide was reconstituted in normal saline and administered at a dose of 50 mg/m^2/day as a continuous infusion for 5 days. Treatments were repeated every 28 days. Dose escalations of 10 mg/m^2/day were made if the preceding wbc count nadir was ⩾ 3000/mm^3 and platelet count nadir was ⩾ 100,000/mm^3. Treatment was continued as long as the patient had stable or responding disease without intolerable toxic effects. The National Prostate Cancer Project criteria for response and progression of disease were utilized (4).

RESULTS

The patient characteristics are shown in table 1. None of the patients entered in the study had objective evidence of tumor regression. One patient was lost to follow-up after his first 5-day infusion, and therapy was discontinued after two cycles in another patient because of an inability to get transportation to the hospital. The other 19 patients were taken off the study because of objective evidence of progressive disease. The median number of cycles administered was two (range, one to five). The median time to disease progression after beginning etoposide was 63 days (range, 31-141). Only one patient experienced a "subjective benefit" attributable to etoposide administration (time to disease progression, 141 days).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	21
Median age in yrs (range): 65 (53-85)	
Performance status*	
1	9
2	9
3	3
Median mos from diagnosis of prostate cancer (range): 33 (15-156)	
Prior therapy	
Radiation	17
Hormone manipulation	21
Cytotoxic†	7
Evidence of progressive disease during etoposide therapy (19 evaluable patients)	
Change in performance status (⩾ 1 level)	19
New bone lesion (scan)	11
Increasing acid phosphatase	12
Increasing soft tissue mass	4

*Eastern Cooperative Oncology Group criteria; median performance status = 2.

†Doxorubicin, 3 patients; estramustine, 3; and 5-FU, doxorubicin, and mitomycin (FAM), 1.

Toxic Effects

Toxic effects were mild. Alopecia was frequently seen. Gastrointestinal upset was mild and easily managed with

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antiemetics. No soft tissue damage from drug extravasation was encountered. There were no instances of documented granulocytopenic infection or thrombocytopenic bleeding. Eleven of 21 patients experienced etoposide-induced myelosuppression with a wbc count $\leq 2500/mm^3$ and/or a platelet count $< 100,000/mm^3$.

DISCUSSION

In this phase II study with etoposide administered as a continuous infusion, we saw no activity in patients with advanced prostate cancer. With no responses in 19 evaluable patients, there is a 98% chance that the true response rate is < 20% (binomial distribution). Although higher doses of etoposide have been reported to be tolerated when given by continuous infusion, the degree of myelosuppression seen in this study does not suggest that substantially higher doses of etoposide would be tolerated in elderly men with advanced prostate cancer. Further evaluation of etoposide in advanced prostate cancer does not appear warranted.

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Phase II Study of Spirogermanium in the Treatment of Metastatic Breast Cancer[1]

Kavitha Pinnamaneni,* Hwee-Yong Yap, Sewa S. Legha, George R. Blumenschein, and Gerald P. Bodey[2,3]

Spirogermanium is an azaspirane-germanium compound that has been shown to inhibit protein synthesis (1). It has a cytotoxic effect on HeLa cells and cells from human malignant lymphoma in vitro, and moderately inhibits the growth of mammary carcinoma 13762 and prostatic carcinoma 11095 in rats (2). In phase I trials, spirogermanium had no myelosuppressive activity, and CNS toxicity was the dose-limiting side effect. Delivery of this drug by continuous infusion made the drug more tolerable to patients and permitted administration of higher doses of up to 200-300 mg/m^2 over 24 hours (3). These considerations led us to initiate a phase II study using spirogermanium in patients with metastatic breast cancer.

MATERIALS AND METHODS

Twenty-one patients with documented metastatic breast cancer were entered in this study. The starting dose of spirogermanium was 200 mg/m^2, administered by continuous infusion 24 hours a day for 5 days and repeated every 2 weeks. In nine patients, because of the absence of dose-limiting toxic effects, we were able to escalate the dose to 250 mg/m^2/day. Two patients known to have disease of the CNS and liver received only 180 mg/m^2 each as a starting dose. The pretreatment characteristics of these patients are shown in table 1.

RESULTS

Two patients died of disease progression on Days 9 and 12 of chemotherapy. Another two patients received only one course each, because they decided against any further therapy. For the 17 evaluable patients, no complete or partial responses were observed. Four patients had no change in disease status for 6-8 weeks and 13 patients had progressive disease. Spirogermanium given by continuous infusion was well-tolerated. No myelosuppression was observed. Eighteen patients had reversible neurotoxic effects, three had mild toxic effects, and 15 experienced moderate neurotoxic effects. The incidences of individual gastrointestinal and CNS side effects are

TABLE 1.—Patient characteristics

Total No. of patients	21
Total evaluable patients	17
Median age in yrs (range)	55 (29-66)
Menopausal status *	
Premenopausal	4 (19)
Postmenopausal	9 (43)
Surgical menopause	8 (38)
Estrogen receptor status *	
Negative	11 (52)
Unknown	10 (48)
Median disease-free interval in mos (range)	16 (0-132)
Median No. of metastatic sites (range)	2 (1-5)
Dominant site of disease *	
Visceral	10 (48)
Soft tissue	5 (24)
Osseous	6 (28)
Median Zubrod performance status (range)	2 (0-4)
Median No. of prior chemotherapeutic regimens (range)	3 (2-6)
Prior hormonal therapy *	11 (52)
Median No. of prior hormonal therapies (range)	2 (1-3)
Toxic effects *	
Gastrointestinal	8 (38)
Nausea and vomiting	7 (28)
Anorexia	3 (14)
Neurologic	18 (86)
Nervousness	6 (33)
Paresthesias	9 (50)
Visual disturbances	10 (55.5)
Extreme weakness	4 (22)
Lightheadedness	5 (28)

* Values = No. of patients (%).

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[3]We thank Miss Ozella E. Rabb for assistance in the preparation of this manuscript.

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shown in table 1. There were no instances of life-threatening toxic effects encountered.

DISCUSSION

This phase II trial failed to identify any activity of spirogermanium given at a dose range of 200–250 mg/m^2 by continuous infusion 24 hours a day for 5 days to patients with metastatic breast carcinoma who had been heavily pretreated. The response rate was < 15% at a 95% confidence limit. Similar results were observed by Budman et al (4) with 30 patients who received intermittent bolus doses of spirogermanium. In contrast, a 25% response rate was reported recently by Falkson and Falkson (5) in patients who had been less heavily pretreated. This difference in response rate may be related to the different dose-schedule rate we employed or, more likely, it may be due to different patient selection factors in their study, such as less extensive prior therapy and better performance status. With the dose used in this study, side effects were tolerable. Efforts to increase the dose did not influence the response rate. We believe that 200–250 mg/m^2 of spirogermanium administered by continuous infusion 24 hours a day for 5 days had no activity against metastatic breast carcinoma in patients who previously had been exposed to multiple chemotherapeutic regimens.

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Multicenter Phase II Trial With 5-Day Continuous Infusion of Vindesine in Metastatic Malignant Melanoma[1]

X. Fabregat Mayol,[2,*] J. Beltran,[3] R. Rubio-Bazan,[4] J. Rifa,[5] R. Rosell Costa,[6] and J. J. Lopez Lopez[7]

Several trials have pointed out a favorable therapeutic index for vindesine or desacetyl vinblastine amide sulfate (DVA) in several human tumors including malignant melanoma (1-3). According to these data, our concern has been to evaluate the activity of DVA administered as a continuous 5-day infusion in disseminated malignant melanoma.

PATIENTS AND METHODS

A total of 15 patients with measurable lesions of metastatic malignant melanoma were eligible for this study. Their characteristics are shown in table 1. Eleven patients had previously been treated with other cytostatic drugs. DVA was administered by iv continuous infusion for 5 consecutive days at a dose of 1.5 mg/m^2/day. World Health Organization criteria of response were used.

TABLE 1.—Characteristics of 15 patients evaluable for response

Characteristic	No. of patients
Median age in yrs (range): 54 (37-69)	
Median Karnofsky performance status (range): 70 (50-80)	
Men	4
Women	11
Median No. of courses given (range): 3 (1-5)	
Prior chemotherapy	11
Site of metastasis	
Nodes	8
Soft tissues	8
Lung	4
Liver	3
Bone	2
Multiple	8
Disease progression	15
Toxicity	
Hematologic	0
Neurologic	2
Paresthesias	2
Constipation	2
Paralytic ileus	2
Myalgia	1
Nausea and vomiting	4
Fever	4
Phlebitis	3
Alopecia	3
Stomatitis	2

RESULTS

Of the 15 patients eligible and evaluable, none showed an objective response. These findings preclude a < 15% response rate with 95% confidence. Hematologic toxicity did not occur. Mild nausea and vomiting, as well as fever and phlebitis, were the most common side effects observed. Alopecia was seen in three patients. Two cases of paralytic ileus warranted discontinuation of the study.

DISCUSSION

In several reports (1,3,4), DVA has shown some efficacy in therapy for malignant melanoma. Our goal of achieving a better therapeutic index by means of a continuous 5-day infusion as the basis for other studies (2,5) has not been reached. Significant myelosuppression was not observed. Phlebitis and fever, as well as nausea and vomiting, were the most common side effects.

In conclusion, we believe that continuous iv infusion with DVA is inactive in previously treated metastatic malignant melanoma. At this dose and schedule of administration the toxicity is acceptable.

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NCI-INSERM Cooperation in Basic and Clinical Cancer Research

The US National Cancer Institute (NCI) and the French Institut National de la Santé et de la Recherche Médicale (INSERM) jointly sponsor an agreement to promote cooperation in basic and clinical cancer research. Limited funds are now available for this program to provide travel expenses and subsistence allowance for US scientists to work in France for periods of up to 1 year on appropriate collaborative research projects with French colleagues. In addition, similar funds are available to pay the living expenses for French scientists to work in the United States on collaborative cancer research projects with American colleagues. Each request for support will be reviewed for scientific merit by the American and French program committees. The deadline for applications for this year is *October 31, 1984.*

For further information, contact: Dr. Bruce Chabner, Director, Division of Cancer Treatment, National Cancer Institute, Bldg 31, Rm 3A52, Bethesda, MD 20205; *or* Dr. Christine Chirol, Hôpital Cochin, Bâtiment Gustave Roussy, 7e étage, 27 rue du Faubourg St. Jacques, Paris 14e, France.

Annual Bekales Prizes and Special Awards

The Bekales prizes and special awards are presented by the Bekales Foundation to scientists in Belgium, Luxembourg, or The Netherlands who are working in the field of cardiology or leukemia.

Bekales prizes (500,000 BF) will be presented to young scientists (< 40 years of age) who have especially distinguished themselves by their research work.

Bekales special awards will be presented to a person (or group of no more than three people) considered to have made outstanding contributions in the field of cardiology or leukemia.

Applications should be sent before *November 15, 1984,* to: Bekales Awards, Fonds National de la Recherche Scientifique, 5 rue d'Egmont, B 1050, Brussels, Belgium.

Cincinnati Cancer Conference III

Cincinnati, Ohio, November 2-3, 1984

Lung cancer is the focus of this conference to be held at the Hyatt Regency in Cincinnati.

For further information, contact: Thomas J. O'Connor, Medical Staff Education, Bethesda Hospitals, 619 Oak St, Cincinnati, OH 45206 (telephone: 1-513-569-6337).

Innovative Cancer Chemotherapy for Tomorrow

New York, New York, November 7-9, 1984

The sixth biennial symposium of the Chemotherapy Foundation will be held at the Barbizon Plaza Hotel under the auspices of the Division of Medical Oncology of the Department of Medicine, the Department of Neoplastic Diseases, and the Page and William Black Post-Graduate School of Medicine, the Mount Sinai School of Medicine (CUNY). Dr. Ezra M. Greenspan, Clinical Professor of Medicine (Oncology), is chairman of the 3-day meeting, which will also include recent developments in chemoimmunotherapy and biological response modifiers. Symposium registrants are eligible for approximately 20 AMA Category I credits.

For further information, contact: Director, the Page and William Black Post-Graduate School of Medicine, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029 (telephone: 212-650-6772).

Workshop on Practical Approaches to Oncology

Fargo, North Dakota, November 9, 1984

This workshop will be held at the Fargo Holiday Inn. Lectures will focus on new trends in management of cancer patients. Current concepts and recent advances in pain management and research will be reviewed, with emphasis on the clinical assessment of the patient with chronic cancer pain and the use of narcotic analgesics in managing pain.

For further information, contact: Medical Education, St. Luke's Hospital, 5th St N at Mills Ave, Fargo, ND 58122 (telephone: 701-280-5933).

1984 Urologic Tumor Symposium

New York, New York, November 15–17, 1984

The latest information on diagnosis and management of renal cell cancer, upper tract urothelial tumors, bladder tumors, prostatic adenocarcinoma, and germ cell testis tumors will be presented at this Memorial Sloan-Kettering Cancer Center postgraduate symposium. Special lectures on oncogenes and monoclonal antibodies are also planned. The course director is Dr. Willet Whitmore; the program has been approved for 23 hours of credit, Category I.

For further information, contact: Continuing Medical Education Planning Office, C-180, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021 (telephone: 212-794-6754).

Sixth International Symposium on Prevention and Detection of Cancer

Vienna, Austria, November 26–29, 1984

Sponsored by the International Society for Preventive Oncology, World Health Organization, Austrian Cancer Society-Austrian Cancer League, and Association of Clinical Scientists-USA, the program for this symposium includes overview lectures, panels, poster sessions, scientific exhibits, and special workshops designed for critical appraisal of current data. Discussions are to concentrate on actions to be taken and on the implementation of existing knowledge for effective cancer control by primary and secondary prevention. Reports are to present progress in multifactorial etiology of oncogenesis, molecular biology, identification of high-risk groups, tumor susceptibility, and clinical and laboratory manifestations of cancer including tumor markers. Participants include the clinician, epidemiologist, pathologist, experimental oncologist, immunologist, socioeconomist, educator, and members of the health care team.

Abstracts and the program will appear in *Cancer Detection and Prevention*, 1984, vol 7, No. 6. Awards for outstanding investigation(s) presented by ISPO. Accredited for 32 CME credit hours.

For further information, contact: Prevention and Detection of Cancer, AMEX POB #790459, Dallas, TX 75379 [telephone: 1-800-527-0297; outside Europe (usa-214) 392-3663; in Europe (austria 43-222) 52-0544].

Second International Symposium on the Modulation and Mediation of Cancer by Vitamins and Micronutrients

Tucson, Arizona, February 12–15, 1985

This symposium will be held at the Arizona Health Sciences Center under the sponsorship of the University of Arizona Cancer Center. Dr. Frank L. Meyskens (University of Arizona) and Dr. Kedar N. Prasad (University of Colorado) will serve as cochairmen. Abstract deadline is *November 1, 1984.*

For more information, contact: Mary Humphrey, Conference Coordinator, University of Arizona Cancer Center, Tucson, AZ 85724 (telephone: 602-626-6044).

Sixty-seventh Annual Meeting of the American Radium Society

Acapulco, Mexico, April 27–May 1, 1985

For information contact: Ms. Suzanne Bohn, Executive Secretary, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

Third European Conference on Clinical Oncology and Cancer Nursing

Stockholm, Sweden, June 16–20, 1985

The scientific program of this conference will emphasize a multidisciplinary approach to the study and treatment of cancer and caring for patients. The program will include posters and free papers, workshops, plenary and teaching lectures, symposia, satellite symposia, and "Ask the Specialist" sessions. All sessions will be held at the Stockholm International Fairs, "Massan."

The deadline for registration and receipt of abstracts is *January 10, 1985.* The registration fee is SEK 2100 (SEK 1100 for registered nurses, radiologic technicians, and medical secretaries). There will be an additional charge for late registration.

For further information, contact: Mrs. Ira Thilén, Stockholm Convention Bureau, Jakobs Torg 3, S-111 52 Stockholm, Sweden (telephone: 08/23-09-90).

Position Vacancy: Associate Director, Biological Response Modifiers Program

The Division of Cancer Treatment, National Cancer Institute, is seeking an Associate Director for its Biological Response Modifiers Program. The Associate Director has overall responsibility for the planning, direction, evaluation, and coordination of basic and applied research on interferons and other biological response modifiers. The incumbent establishes program priorities; allocates resources; evaluates program effectiveness; and administers a $20 million contract and grant program and a staff of approximately 103 scientific and support personnel. The salary range is $58,938–$66,400 (physicians may be eligible for Physicians' Comparability Allowance up to $10,000/year).

Applications must be postmarked no later than *September 30, 1984.*

Applications and requests for further information should be addressed to: Cynthia A. Kauff, PMS, National Cancer Institute, Bldg 31, Rm 3A08, Bethesda, MD 20205 (telephone: 301-496-6503).

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